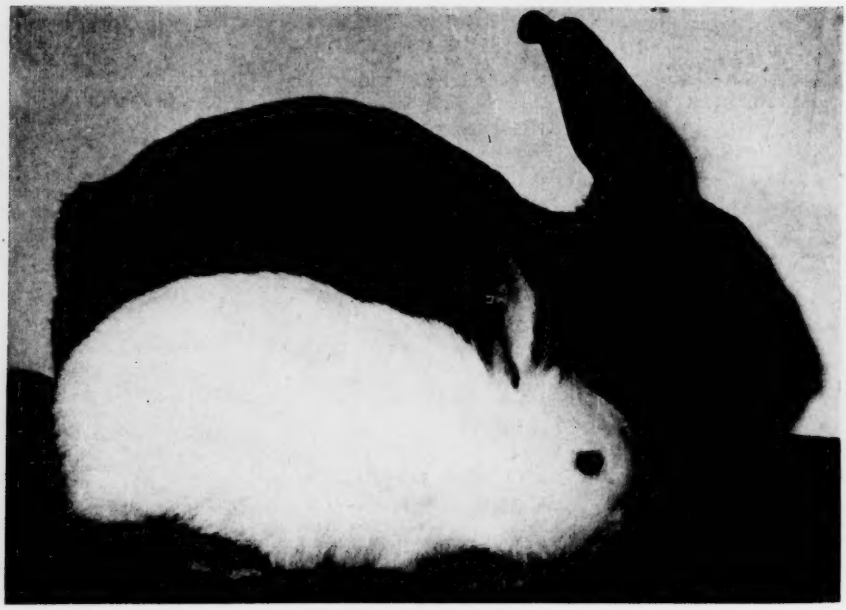
The Journal of HEREDITY



LONG RANGE FOSTER MATERNITY

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Anatomy of Heterochromatin

More on the Human Sex Ratio

Spermatogenesis in the Domestic Fowl

The Genetic History of the Arabian Horse

Obese Mice—Sterile Mothers and Fertile Fathers

VOLUME 45 MARCH-APRIL, 1954 NUMBER 2

The American Genetic Association

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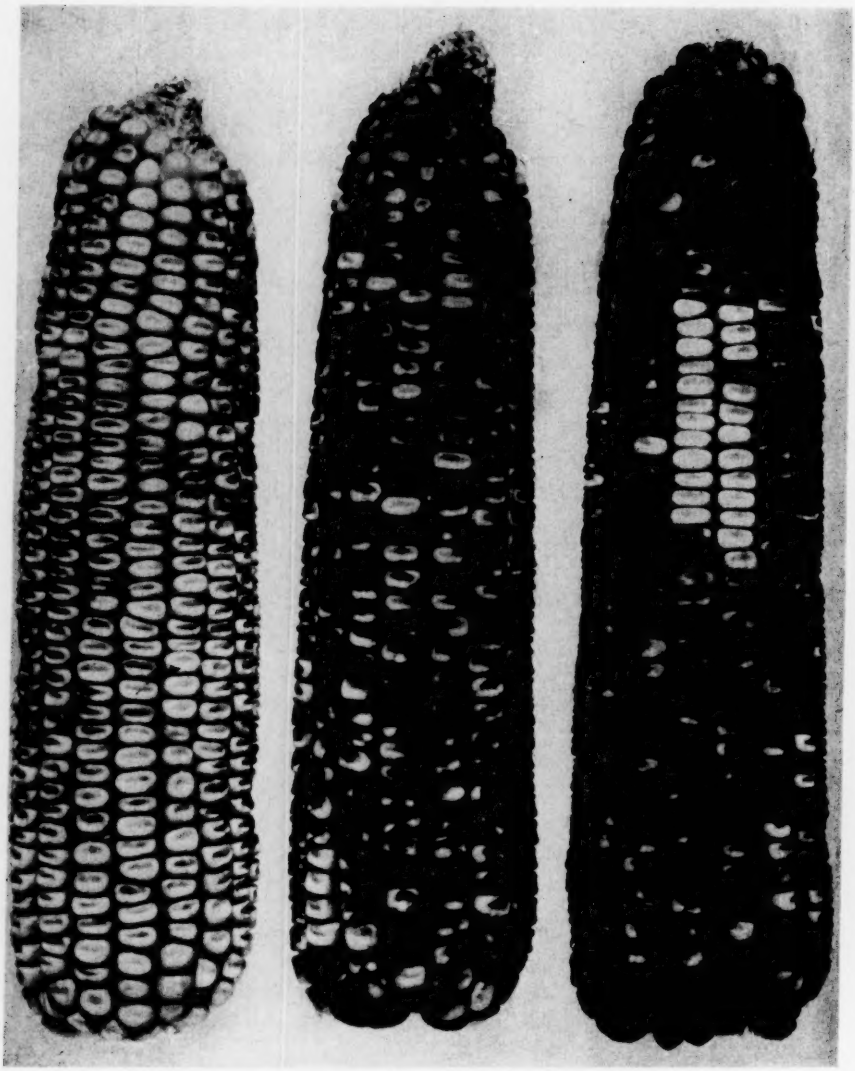

MAIZE EARS SHOWING DIFFUSE PERICARP

Figure 1

Diffuse is epistatic to red pericarp and cob and suppresses the pigmentation in an irregular fashion over the ear. The color differences within an ear are not heritable. The phenotype varies from plant to plant, however, from light (left) through intermediate (middle) to dark diffuse (right) depending, presumably, on modifying genes.

DIFFUSE, A PATTERN GENE IN ZEA MAYS

Epistatic to Red Pericarp

R. A. Brink and Irwin M. Greenblatt*

THE genetic classification of maize pericarp colors formulated earlier by Emerson and particularly by E. G. Anderson (summarized in Emerson, Beadle and Fraser[1]) included all the widely occurring types. A few less frequent forms have come to light since. A recently discovered one is diffuse, the subject of the present report.

Diffuse is represented in our cultures by the descendants from two ears collected separately in Peru. We are indebted to Dr. Paul Mangelsdorf, Harvard University, for seed of the wide assortment of pericarp types of Latin-American origin in which the two examples of diffuse were found. The new phenotype has not been encountered in stocks from any other source, and probably is rare.

Superficially, diffuse might be mistaken for one or another of the widely variant forms of mosaic pericarp.[2] The two phenotypes, however, are usually distinct; they are referable to separate loci; and the color patterns on mature ears are differently related to the respective causal genes.

Typical diffuse ears are illustrated in Figure 1. The over-all effect of diffuse is commonly an irregular patchwork of deeply colored, lightly pigmented, and colorless areas. Color occurs in all cell layers of the pericarp. A distinctive feature of this type of maize, however, is the intergradation of tones between small adjacent dark and light pericarp areas. The boundaries between the colored and colorless patches are not sharply delineated, as regularly occurs on variegated and often on mosaic ears, but are indistinct. One is reminded of the "running effect" in a water-color painting beyond the area to which the brush has been applied, hence the name diffuse.

Most of the diffuse ears which we have grown are of the dark type shown at the right in Figure 1. So-called light phase ears (pictured at the left) and intermediates (middle) possibly as a distinct class, also occur. The relationship between these sub-types is not yet clear. A few families have been seen in which the distribution of plants with light and dark ears suggested the action of a major modifying gene.

The full expression of the diffuse pattern is associated with the presence of the P^{RR} gene (red pericarp, red cob) at the P locus in chromosome 1. Colorless patches occur on such ears in corresponding positions on pericarp and cob. The diffuse effect is displayed also in the husks of P^{RR} plants in which this tissue is pigmented. The diffuse pattern is shown only on the cob of plants carrying the P^{WR} (colorless pericarp, red cob) allele. Classification for diffuse on $P^{WR}P^{WR}$ plants is sometimes difficult or even impossible because the areas of reduced pigmentation on the cob often are small, infrequent, or lacking in contrast with the normal red background. Similarly, the diffuse effect is superimposed on the irregular pericarp and cob pattern conditioned by the mutable P allele, variegated (P^{VV}). Diffuse on a (P^{MM}) ear cannot be recognized with certainty unless the plant is heterozygous for red cob.

As shown in Table I, plants heterozygous for diffuse mated with homozygous red pericarp, red cob, non-diffuse individuals give equal numbers of diffuse and red pericarp, red cob offspring. Heterozygous diffuse

*University of Wisconsin, Madison 6, Wisconsin. Paper No. 538 from the Department of Genetics, College of Agriculture. This work was aided by grants from the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation. We wish to express our appreciation to Ronald E. Anderson and Carl M. Campbell who assisted in the breeding work and to Eugene Herrling who made the photograph appearing in Figure 1.

plants, heterozygous at the *P* locus for red pericarp, red cob and colorless pericarp, red cob (*PRR*/*PWR*) pollinated with the ordinary *PWR* type, which is non-diffuse, yield with equal frequency four classes of plants as shown in the last line of Table I. It is evident from these data that diffuse is distributed independently of the *P* alleles and acts as a dominant partial inhibitor of the *P*-series of ear color effects. The symbols *Df* and *df* are proposed for diffuse and non-diffuse, respectively.

One family segregating *Df*, *PRR*, *PWR*, and a weakly colored pericarp type not in the *P*-series has been seen. Diffuse was recognizable on red-cobbed ears of the latter class. Pigmentation of the pericarp over the light patches on these cobs was reduced. This fact shows that the modifying effect of diffuse on pericarp color is not limited to the *P*-series of phenotypes.

A series of linkage tests against reciprocal translocations generously supplied by Dr. E. G. Anderson, California Institute of Technology, shows that the *Df* locus is in either the distal portion of the long arm of chromosome *2* or the long arm of chromosome 4. The F_1 hybrids between diffuse and the respective translocations were mated with colorless pericarp, red cob (*PWR*) plants. The latter were used in the test cross because we were unaware at the time that *PRR* must be present in order that *Df* be fully expressed in the phenotype. The *PWR* segregates in the resulting families were discarded as not regularly classifiable for diffuse, and only the *PRR* plants were used in the linkage determinations.

As the data in Table II show, diffuse gives about five percent recombination with T2-4b. According to Anderson and Longley (cited from Maize Genetics Cooperation News Letter 26, 1952) the chromosome breakage points in this reciprocal translocation are located at 2L.88 and 4L.54. All the other reciprocal translocations used involving breaks

TABLE I. Inheritance of diffuse in relation to two alleles, red pericarp, red cob (P^{RR}) and colorless pericarp, red cob (P^{WR}), at the *P* locus

Family	Parent genotypes	Number of offspring			
		Diffuse		Non-Diffuse	
		P^{RR}	P^{WR}	P^{RR}	P^{WR}
12–153	$Df P^{RR}/df P^{WR} \times df P^{RR}$	59	0	49	0
–158	" "	54	0	58	0
–166	" "	41	0	66	0
–152	$Df P^{RR}/df P^{WR} \times df P^{WW}$	31	42	37	38

TABLE II. Linkage tests of diffuse against reciprocal translocations involving chromosomes 2 and 4

Family	Mating	Chromosome markers*	Number of offspring				Percent recombination
			Semisterile		Normal		
			Diffuse	Non-diffuse	Diffuse	Non-diffuse	
12–208	*Df/T1–4a* × *df*	1L.49 4S.66	4	10	10	11	
–209	"		3	8	9	12	
–210	"		16	5	22	6	
–211	"		13	7	12	14	
Total			36	30	53	43	48.8
12–219	*Df/T2–4l* × *df*	2L.56 4S.51	23	18	23	24	53.4
12–221	*Df/T2–4b* × *df*	2L.88 4L.54	0	30	16	0	
–222	"		3	25	33	3	
–223	"		1	14	14	0	
Total			4	69	63	3	5.0
12–224	*Df/T2–6b* × *df*	2S.69 6L.49	8	9	10	10	
–225	"		21	16	12	19	
–226	"		7	10	9	10	
Total			36	35	31	39	53.2
12–227	*Df/T2–7c* × *df*	2L.48 7S.50	10	12	5	11	
–228	"		7	13	6	4	
–229	"		8	6	8	3	
–230	"		8	9	9	6	
Total			33	40	28	24	45.6

* Cytological determinations by E. G. Anderson and A. E. Longley. Cited from Maize Genetics Cooperation News Letter 26, 1952.

in either chromosome 2 or chromosome 4, or in both, T1-4a (1L.49, 4S.66), T2-41 (2L.56, 4S.51), and T2-7c (2L.48, 7S.50), assort independently of diffuse. These additional data definitely exclude the mid-regions of the long arm of chromosome 2 and the short arm of chromosome 4 as possible sites of the *Df* locus. They do not permit a decision, however, between the two possible locations of *Df* indicated by the close linkage with T2-4b, namely, the distal portion of the long arm of chromosome 2 and the long arm of chromosome 4.

Several hand pollinated diffuse ears have been obtained which contained relatively large patches of kernels with near-colorless pericarp (see ear at the right in Figure 1). This fact suggested at first that *Df* was an unstable gene which, like variegated (*PVV*) and mosaic (*PMM*), mutated somatically to different levels of expression which were then repeated in the offspring. Diffuse, however, is different. Plants grown from the kernels in the light patches regularly bear ears having the same diffuse pattern with respect to the number and distribution of dark and light areas as plants reared from adjacent dark diffuse kernels. The data bearing on this question are assembled in Table III.

Pairs of families were grown from the light and dark seed on seven ears each carrying a relatively large patch of near-colorless kernels. Five of the pistillate parent plants were homozygous *PRR* and two were heterozygous *PRRPWW*. All were pollinated by *PWR*. As the progeny data in Table III show, the light kernels from the first five ears yielded 41 diffuse and 44 non-diffuse, and the corresponding dark kernels gave 57 diffuse and 58 non-diffuse, plants. There was no detectable difference in the expression of diffuse within pairs of families; light and dark kernels alike transmitted dark diffuse. The results with ears six and seven were the same except that an additional class of offspring, colorless pericarp red cob, was present in these families. This latter class was not scored in detail for diffuse but the presence of the character in it was disclosed by the occurrence of conspicuous light colored patches on some of the cobs.

Thus there is no evidence that the prominent light colored areas on otherwise dark diffuse ears are the result of gene mutation. These gross variations in the expression of *Df* appear to be purely somatic.

Summary

1. Diffuse (*Df*) is a dominant gene in maize which partially inhibits pigmentation in pericarp and cob. Its maximum expression is attained in combination with *PRR*, self red pericarp and cob, to which it is epistatic. Manifestation of the *Df* effect is correspondingly reduced in plants carrying *P* alleles lower in the dominance series, such as *PWR* and *PVV*, which limit the distribution of pigment.

2. The inhibitory action of *Df* varies locally over the ear from complete suppression of pigmentation to no detectable effect, and thus results in a highly irregular pattern. The transition from a colored and to a colorless pericarp area usually is gradual.

3. The *Df* gene was found in two stocks of Peruvian origin. It has not been encountered in numerous other strains under observation, and probably is rare.

4. The *Df* gene gives about 5.0 percent

TABLE III. Distribution of offspring from kernels in conspicuously light colored patches and dark areas on the same ears

Ear number	Parent genotypes	Kernel color	Progeny		
			$_pRR$		$_pWR$
			Diffuse	Non-diffuse	
1	$_{Df}pRR/_{df}pRR \times _{df}pWR$	Light	1	7	0
		Dark	14	19	0
2	" "	Light	2	1	0
		Dark	8	7	0
3	" "	Light	7	4	0
		Dark	10	5	0
4	" "	Light	8	12	0
		Dark	10	18	0
5	" "	Light	23	20	0
		Dark	15	9	0
6	$_{Df}pRR/_{df}pWW \times _{df}pWR$	Light	12	13	19
		Dark	8	13	20
7	" "	Light	2	6	8
		Dark	9	10	16

recombination with translocation 2-4b which serves to mark chromosome 2 at L.88 and chromosome 4 at L.54. The results of tests against other translocations involving these two chromosomes point to the distal portion of the long arm of chromosome 2 or the long arm of chromosome 4 as the site of the *Df* locus.

5. Kernels from a relatively large patch which is colorless or near-colorless as the result of *Df* action give the same kind of progeny as typical dark diffuse kernels from the same ear. This fact shows that these light colored areas do not result from mutation of the *Df* gene, but are pattern effects.

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1. Emerson, R. A., G. W. Beadle and A. C. Fraser. A summary of linkage studies in maize. *Cornell University Agricultural Experiment Station Memoir* 180:1-83. 1935.

2. Hayes, H. K. Inheritance of a mosaic pericarp pattern color in maize. *Genetics* 2:261-281. 1917.

◆

A NEW EDITION OF SCHRADER'S "MITOSIS"

THE second edition of Schrader's critique of mitosis* follows the general outline of the first edition published in 1944. The post-war research has been integrated with the older information in the revised edition and a concise discussion of the contributions of cytochemistry to the problems of mitosis has been added.

Mitosis is considered in the inclusive sense as any nuclear division involving a spindle apparatus. Meiosis consequently is regarded as a modified mitosis, thus permitting a unified treatment of the problems of chromosome movements. It is apparent that a treatise of 125 pages cannot consider in detail all phases of chromosome structure and behavior. As shown by the title, the author has concentrated largely upon the problems of chromosome movements and therefore does not attempt to review in detail such topics as coiling and number of chromonemata.

The nature and origin of the spindle, the structure and function of the kinetochore and the various hypotheses of chromosome orientation and movement are concisely discussed and critically but impartially evaluated by the author. In the 1944 edition the evidence for and against the reality of spindle fibres was reviewed in detail. The author stated in conclusion, "The question of the reality of spindle fibres may be regarded as settled." Research with the polarization microscope and microdissection studies published since the first edition are discussed in the new edition. These recent studies firmly support the fibrous nature of the living spindle. As a result of recent progress, Schrader concludes that the cytologist is much closer than he was ten years ago to being able to ask specific questions of the physical chemist in regard to forces of contraction and expansion of the spindle.

Attention of the reader is directed to the often neglected topic of chromosome movement during prophase and prior to the formation of the spindle. A chapter entitled "Related Problems" discusses briefly the pairing of homologues, behavior of chromosome ends, heteropycnotic attraction, kinetochore attraction, the nuclear membrane and meiotic pre-metaphase stretch.

Mitosis has not been amenable to inclusive generalizations. The hypotheses of mitosis based upon analogies to individual physical systems have encountered contradictory exceptions. The author emphasizes that mitosis is an extremely complex process involving many mechanisms. The concept of any single basic force underlying mitosis must therefore be regarded as naive in the extreme. Schrader's book may well serve as an experience in scientific maturity for the student initiating research in this field. Although progress has been slow and difficult, Schrader states that the number of new experimental approaches during the past ten years greatly exceeds the number during the first thirty-five years of the century. Since the new attacks are primarily physical and chemical in their direction, he emphasizes the necessity of close contacts among cytologists, biochemists and biophysicists.

Approximately 600 references are cited. The papers listed have with few exceptions been published since 1925. This comprehensive and modern reference list should be of considerable interest to the cytologist and his graduate students.

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**Mitosis*—The Movement of Chromosomes in Cell Division. Second edition. Franz Schrader. Columbia University Press, New York, New York. 1953.

STERILE, OBESE MOTHERS

MEREDITH N. RUNNER AND ALLEN GATES*

A STERILE-OBESE syndrome in mice has been reported by Ingalls, *et al.*[3] Evidence was presented that adiposity can be a familial character in mice; even as in man. If and when parental mice, themselves possessing well proportioned contours, begot offspring with rounded contours, they did so about one-fourth of the time. This adequate fit for a monogenic ratio was *prima facie* evidence that the lean parents were heterozygous and that the overweight offspring were homozygous for a recessive gene causing the sterile-obese syndrome. Since other familial characteristics in mice are known to approximate monogenic ratios in the absence of segregation of a single gene, direct and final proof for the manner of inheritance of this particular syndrome has awaited analysis of offspring from the plump phenotypes.

Motherhood became possible for these otherwise sterile mice by administration of gonadotropic hormones and induction of ovulation. Eggs produced by this procedure were transplanted for gestation in unrelated foster mothers having family histories of characteristically trim figures. Donors of fertilized and unfertilized eggs were female mice that were obviously obese and from the same colony as those reported by Ingalls, *et al.*[3] Mating tests with nine offspring from obese donors showed each egg had indeed received a factor for obesity.

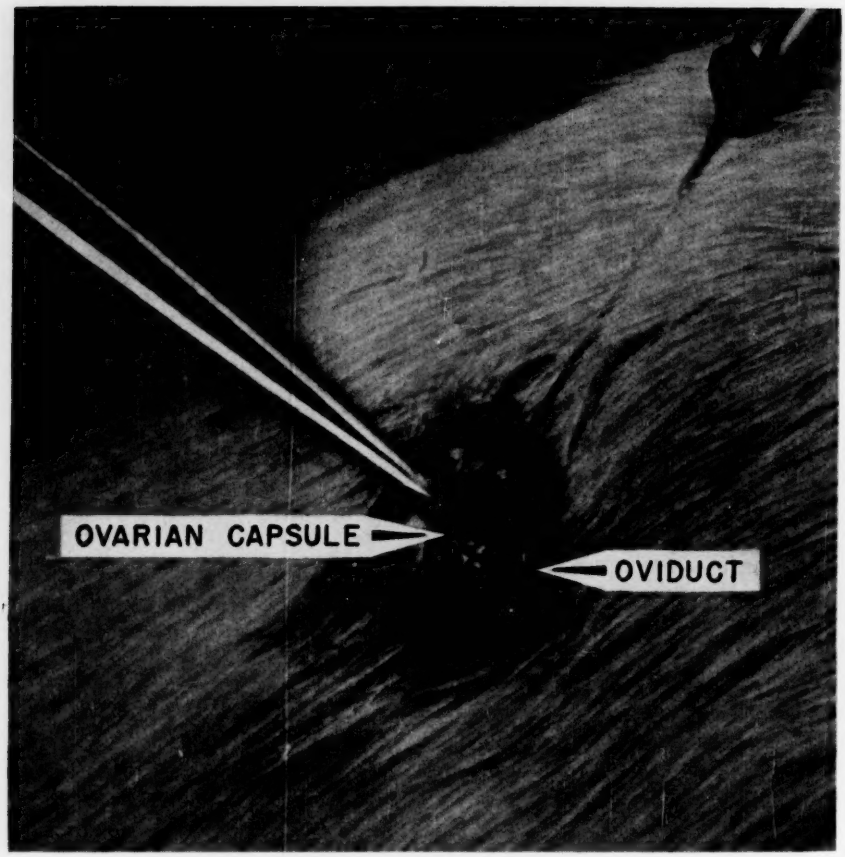
Methods and Materials

Procedures have been reported for obtaining fertilization after transplantation of ova from artificially induced ovulations,[6] and for obtaining fertilization in immature mice subsequent to hormonally induced ovulation and mating.[7] The actual technique of transplantation used for this investigation is illustrated in Figure 2. Both transplanted eggs and eggs native to the foster mother were gestated simultaneously. Young from the transplanted and native eggs were invariably distinguished by genetic tags.† Animals in litters 1, 2, 4 and 5 were identifiable at birth by presence or absence of ocular pigmentation. The young in litter 3 were tagged by coat color.

Three combinations of donors, donor's mates, recipients and recipient's mates were used (Table I). Litters 1, 2 and 3 involved transplantation of unfertilized eggs following artificially induced ovulations in obese donors. Litters 1 and 2 followed transplantation of unfertilized eggs from obese donors having pigmented eyes (*PP*). These eggs were fertilized within a foster mother having non-pigmented eyes (*pp*) by sperm from a male having non-pigmented eyes (*pp*). Native offspring

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor Maine. This investigation was supported in part by a research grant G 3859 from the National Cancer Institute of the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

†Recently Kurbatov reported (*Usp. sovr. Biol.* 31: 300-314, 1951) hereditary change after interchange of fertilized eggs between breeds of rabbits. (He found that 8.3 per cent of 72 young had coat colors not present in their parents or their littermates.) Should this finding be confirmed we are confronted with chaotic inheritance. Since the original report of Heape in 1890, literally hundreds of mice, not to mention large numbers of rabbits, have been reared in this country from transplanted eggs. Each mouse, to date, has conformed to its genetic expectation. The inherited determinants that we have used for tagging gametes are stable mechanisms. Except for the fantastic improbability of mutation at one specific locus and within one particular gamete this system of tagging, in our hands, is entirely dependable.



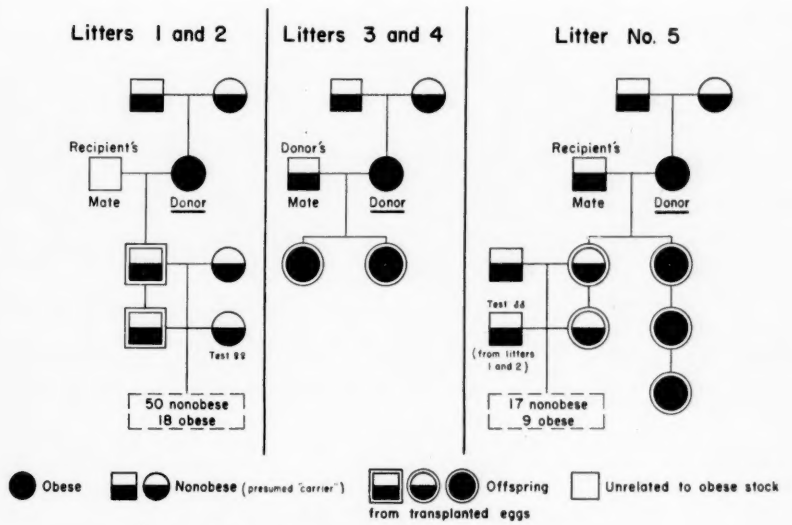
OPERATIVE TECHNIQUE

Figure 2

The method used for transplanting unfertilized eggs is illustrated above. Egg production was induced in a normally sterile obese female. The eggs were transferred to a glass pipette and injected into the ovarian capsule of a non-obese foster mother. The transplanted eggs were gestated along with the foster mother's own progeny.

therefore had non-pigmented eyes (*pp*) and young from the obese donors showed ocular pigmentation (*Pp*). Litter number 3 utilized coat color for tags. Both the obese donor and the recipient's mate were nonagouti (*aa*). The recipient was agouti (*AA*). Consequently native offspring appeared agouti (*Aa*) and young from the obese donors were nonagouti (*aa*). Litters 4 and 5 (Figure 3) followed transplantation of fertilized eggs from obese donors in which ovulation and mating had been induced by hormone administration. The donor and donor's mate had pigmented eyes (*PP*) and the recipient and recipient's mate had non-pigmented eyes (*pp*). Resulting offspring from the recipients' mating had non-pigmented eyes (*pp*) and offspring from the donor's mating had pigmented eyes (*PP*).

Offspring from eggs transplanted from obese donors included both lean



FIVE LITTERS FROM OBESE DONORS

Figure 3

Progeny of sterile obese donors were obtained from five foster litters. In litters 1, 2 and 5, unfertilized eggs were transplanted, and were fertilized by the recipient's mate. In litter 3 and 4 fertilized eggs were transplanted. The origin of the resulting mice was established by markers—(coat color and eye color). Breeding tests of the foster progeny in litters 1, 2 and 5 proved that a recessive gene for obesity had been transmitted.

and fat individuals. The corpulent young were themselves evidence that the donor had transmitted to them a factor for obesity. The thin offspring from obese donors were subsequently paired with a mate known to have produced obese young in order to detect whether the transplants had received within their ova a factor for obesity. Should all ova from obese donors carry a factor for obesity one would have unequivocal evidence for the monogenic recessive nature of this obesity.

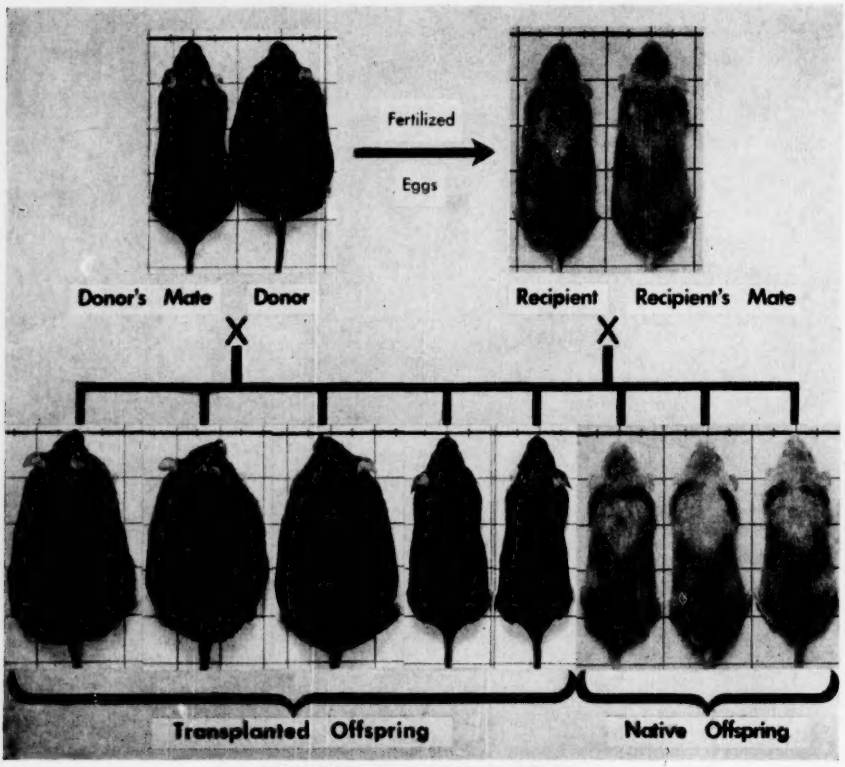
Results and Discussion

Sixteen young mice were obtained from eggs transplanted from obese donors. Nine of these (Figure 3) survived sufficiently long to be identified relative to obesity. Young mice descendant from obese donors have been obtained from five litters (Table II). Litters 1 and 2 each included one male with ocular pigmentation hence derived from transplanted eggs. These male offspring from obese mothers were well proportioned and were crossed with females that had obese offspring from a previous mating. The two males from transplanted eggs sired 12 litters having a total of 68 offspring, consisting of

TABLE I. Genetic tagging of offspring from transplanted eggs

Litter	Type of Eggs Transplanted	Recipient	Genetic Markers			Diagnosis of Offspring	
			Recipient's Mate	Obese Donor	Donor's Mate	Transplant	Nontransplant (Native)
1 and 2	Unfertilized	*pp*	*pp*	*PP*	.....	*Pp*	*pp*
3	Unfertilized	*AA*	*aa**	*aa*	.....	*aa*	*Aa*
4 and 5	Fertilized	*pp*	*pp*	*PP*	*PP**	*PP*	*pp*

*These males had previously sired obese young.



ONE LITTER—FOUR PARENTS

Figure 4

Litter No. 5 was derived by transplantation of fertilized eggs. The eight progeny were all borne in one litter by the recipient female. The five black animals were derived from foster eggs—the three light colored mice were the recipient's own offspring. Three of the black offspring promptly became overweight and thereby demonstrated that the obese donor mother had transmitted the trait to at least three of five descendants. The remaining two foster females had trim figures.

18 obese and 50 non-obese. Thus the two males from obese donors, although their sire was from a non-obese stock, each contained a latent determinant for obesity.

Litters 3 and 4 involved transplantation of eggs from obese donors and fertilization by sperm from males known to have produced obese young. Each litter included one female that was genetically tagged as descendant from an obese donor. These two offspring became excessively chubby at four weeks of age thereby indicating that both of the eggs, as well as the fertilizing sperm, had factors for obesity. Again the obese donors had transmitted obesity to their descendants.

Litter number 5 (Figure 4) was derived by transplantation of fertilized eggs. The obese donor had been artificially induced to ovulate and mate with a male known to have produced obese young. Five female young tagged by ocular pigmentation were born and reared. Three of these promptly became overweight and thereby demonstrated that the obese donor mother had transmitted the trait to at least three of five descendants. The remaining two females from transplanted eggs had trim figures. If they carried but one recessive determinant for obesity they could have received such from either parent since the donor was phenotypically obese and the sire had previously produced obese offspring. These females were mated with males from litters 1 and 2, that had normal contours and were likewise derived from eggs transplanted from obese mothers. Two litters from each female gave 26 young. Combined data

gave nine obviously fat and 17 non-obese young; sufficient evidence that the young from obese donors were capable of transmitting an obese trait which they might have received from their obese mother.

Obesity in humans frequently has been attributed to abnormal eating habits caused by psychogenic factors. The obesity investigated here is also accompanied by excessive food intake. The possibility of psychogenic influence as a cause of this obesity in mice can be dismissed. Obesity and hyperphagia were transmitted through the ovum from mother to offspring in spite of the fact that the offspring were gestated and reared by unrelated, normally proportioned foster mothers.

The technique of transplantation of eggs has served as an adjunct to genetic analysis by testing sterile and presumed genetic recessive donors. Quasi monogenic ratios have frequently been reported in mice. Chase[1] recorded occurrence of polydactyly, Green[2] reported sacralization of vertebra 26, Murray and Green[4] reported frequency of abdominal spotting and Reed[5] reported analysis of harelip. All of these traits have approximated monogenic ratios but none was found to be governed by a simple hereditary unit. Suffice to summarize that when animals in the 25 percent category were bred *inter se*, the frequency of manifestation of the traits failed to approach the 100 percent expectation. The critical proof of the monogenic nature of the 3:1 ratio must depend therefore upon either dividing the phenotypically normal animals into the one-third dominant homozygotes, and two-thirds heterozygotes, or showing that the recessive, in our case obese, animals are homozygous. If the obese female is truly recessive, as the Mendelian hypothesis states, then each egg must carry an obese gene. Each offspring from her ova therefore must have at least one obese gene; the other genetic complement having come from the male will codetermine the phenotype of the individual.

Since 100 percent of offspring from obese animals were found to be carriers for the characteristic, the present data exclude interpretation of a quasi monogenic condition and establish the unit recessive inheritance of obesity in this particular family of mice.

Summary

Offspring have been obtained from sterile-obese female mice following hormonally induced ovulation and transplantation of resulting eggs to fertile recipients. Analysis of experimentally produced offspring has shown that each of nine offspring from obese donors was either corpulent itself or a transmitter of corpulence. Psychogenic origin of this familial adiposity has been precluded since it has been shown that obesity was transmitted within the egg cell in spite of fertilization within and nurture by normally proportioned foster mothers. The present data therefore augment the monogenic ratio observed by Ingalls, *et al*[3] by showing that the overweight mothers endowed each ovum with an inherent propensity for obesity.

[Since our manuscript was submitted, Lane and Dickie have bred offspring from additional obese mice that were cleverly streamlined by rigid dietary regime. Unfortunately their observations do not satisfy our criteria for demonstration of unit gene inheritance. We are pleased however, to receive partial confirmation of our findings insofar as experimental measures can offset the secondary sterility portion of the obesity syndrome. Both projects have provided parenthood for genetically obese, and otherwise completely sterile mice.]

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TABLE II. Analysis of offspring from transplanted eggs

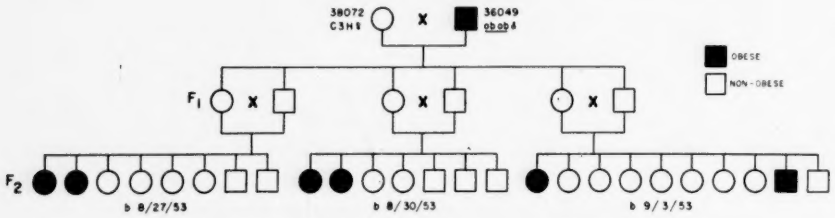
Litter number	Number and type of offspring		Phenotypes of young from obese donors		Results from test matings of nonobese transfers		Genotype of offspring from obese donors
	Transplanted	(Native)	Obese	Nonobese	Obese	Nonobese	
1	1 ♂	(3)	—	1	10	28	1 *Ob ob*
2	1 ♂	(1)	—	1	8	22	1 *Ob ob*
3	1 ♀	(6)	1	—	—	—	1 *ob ob*
4	1 ♀	(1)	1	—	—	—	1 *ob ob*
			3	—	—	—	
5	5 ♀	(3)	—	2	5	8	3 *ob ob*
					4	9	2 *Ob ob*
Total	9		5	4	27	67	

(*Received September 28, 1953*)

FERTILE, OBESE MALE MICE

Relative Sterility in Obese Males Corrected by Dietary Restriction

PRISCILLA W. LANE AND MARGARET M. DICKIE*



PEDIGREE RECORD OF ONE BREEDING OBESE MALE

Figure 5

Obese male 36049 was kept on a restricted diet and mated successfully with a normal female producing six offspring.

VARIOUS investigators have stated that genetically obese (*obob*) mice are sterile, i.e., have not produced offspring when mated *inter se* or with normal non-obese mice.[1, 2] Because of this sterility it is necessary to mate heterozygous (*Obob*) males and females to produce obese animals. Such matings result in obese animals in a ratio of 1:3 non-obese. The non-obese mice must be tested to determine which are the heterozygotes to be used for matings and thus a large breeding colony has to be maintained in order to produce relatively few obese animals.

Recently, however, obese animals have been experimentally produced by another method. Ovulation has been induced in obese females and these ova have been transferred to normal females and fertilized by sperm from heterozygous males.[3] This particular procedure showed not only that obese ova are viable but that the obese condition is due to a true unit recessive gene and not to multiple factors or modifiers as is the case with obesity in yellow mice. Ova transfer being a complex technique is not a practical means of producing large quantities of obese animals.

If obese males or females could be bred, the testing of heterozygotes would be unnecessary and linkage studies and other experimental work could be more easily conducted. With this thought in mind, a study has been made to see if obese males could be made to breed.

Procedures and Results

Two obese males were sacrificed at five to six months of age. In one male the testes were descended and in the other they were not in the scrotal sacs. Undescended testes and a lack of well defined scrotal sacs seem to be relatively common among obese male mice. The sperm were stripped from the vasa deferentia and although sperm counts were not made, actively motile sperm were present in both animals. This indicates that the testes of the obese males regardless of whether or not they had descended into the scrotal sacs were functioning normally and some factor other than the lack of sperm

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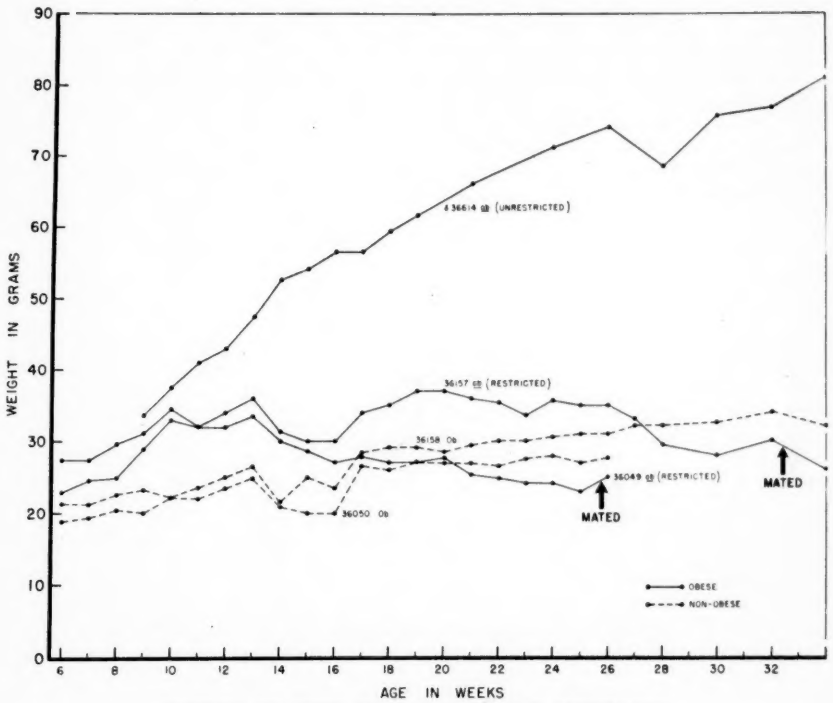

EFFECT OF DIET ON OBESITY AND FERTILITY

Figure 6

The chart above compares the weights of one obese male whose diet was not restricted with obese males put on a restricted diet at six weeks of age, and with non-obese males. The male on an unrestricted diet did not mate, whereas the two males on a restricted diet mated at *26* and *32* weeks of age.

must be responsible for this relative sterility.

Following this observation several obese males, six weeks of age, were put on a restricted diet. These animals were placed two in a pen and were fed approximately three to four grams of Derwood Food or Purina Laboratory Chow per day per animal. Food supply was regulated by the weight of the animal, i.e., animals were weighed weekly and enough food was given daily to maintain them at a weight of *26* to *30* grams, (Figure *6*). At seven months of age two of these obese males which had been restricted in diet and weighing *25* and *26* grams respectively were placed with strain C3H/Di females for several overnight mating periods. No food was available during these mating periods. The outcross to strain C3H/Di, a completely unrelated strain, was made for two reasons. First, to show that each male although he had never been allowed to become fat was genetically obese. This would be proved by the appearance in the F_2 generation of a ratio of one obese to three non-obese. Secondly, should this ratio occur it would offer further proof that the obese condition is due to a unit recessive character and not to modifiers or multiple factors.

These two obese males successfully mated with the C3H/Di females and produced offspring. Obese male *36049* sired six offspring (Figure 5) and obese male *36157* sired seven offspring. These F_1 animals were mated *inter se* and have produced in the F_2 generation 10 obese and 41 non-obese animals.

Another mating of male *36157* was with a female, heterozygous for obesity, who had not at the time of mating been tested. Of the nine offspring resulting from this mating three were obese and six were non-obese. These matings show that some obese males are fertile and when kept at a weight of 25-30 grams by diet restriction will breed successfully. Further work on breeding obese male mice is in progress.

Summary

Obese (*obob*) mice on an *ad libitum* diet

have never bred and produced offspring. These studies have shown: 1) that the testes of the obese males produce motile sperm; and 2) that some obese males will breed when kept on a restricted diet, and not allowed to exceed a weight of 25-30 grams. Further proof is offered that the obese condition is due to a unit recessive gene.

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(*Received December 2, 1953*)

♦

RADIATION EFFECTS ON LIVING SYSTEMS

WITH the rapidly expanded use of radiations that gained impetus with the discovery of nuclear fission and the production of radioactive isotopes in the program of the Atomic Energy Commission, the 1950 symposium on Radiobiology* was indeed timely. The symposium, according to its foreword, was organized to elaborate information along the following four lines: 1) to survey the physical interaction of ionizing radiation and matter; 2) to discuss and elucidate the chemical changes arising from the transfer of their physical energy; 3) to examine the biological effects and 4) to discuss the changes occurring in living tissues. While treating the subject in this light, the primary function of the symposium was not to present new research findings, but rather to summarize the present state of knowledge concerning the various aspects of radiation. The "objectives" comprised fundamental concepts and ideas rather than isolated fragments of information which had not yet matured into their proper relationship with the over-all field of radiobiology.

In the opinion of the reviewer the objectives were accomplished. This was largely due to the excellent choice of speakers; those persons who came from abroad, Drs. Walter Dale, George Hevsey, and Raymond Latarjet, added materially to the success of the symposium. The logical arrangement of the topics discussed, ranging from the physical processes involved in ionizing radiations to the effects of ionizing radiations on cellular metabolism with sections on energy transfer in living material, radiation chemistry, chemical processes in radiobiological reactions, biochemical aspects of ionizing radiations, and the effects of radiation on genetic material, brought together the highlights of our present knowledge of radiobiology.

In addition to the material bearing on the physical and chemical mechanics of radiation, there were several papers of particular interest to geneticists. Dr. Norman H. Giles cited recent work on the mechanism of chromosomal alterations induced by various radiations. It is noteworthy that evidence is accumulating pointing to the idea that many of the cytological effects observed in irradiated material result from the indirect effects of radiation; oxygen seems to play a key role in contributing to an increase of chromosomal damage by radiations. The target theory is undergoing considerable modifications in view of newer facts. The old idea that a single ionization within a chromosome produced a single detectable event appears to be oversimplified, consequently a modification is long overdue. The more recent evidence in this regard points to the view that the "sensitive area" within the cell is much larger than has been supposed; this being the case, factors such as the presence of oxygen in the cellular environment may markedly alter the effects of ionization.

Dr. H. J. Muller gave a comprehensive review of the effects of radiations on the genic level. His remarks were confined entirely to *Drosophila*, the organism with which he has done most of his work. Since there seem to be some differences between the

(*Continued on page* 64)

**Symposium on Radiobiology*—The Basic Aspects of Radiation Effects on Living Systems. Oberlin College, June 14-18, 1950. Edited by James J. Nickson, John Wiley and Sons, Inc., New York.

STUDIES ON THE HUMAN SEX RATIO

4. Evidence of Genetic Variation of the Primary Sex Ratio in Man

MARIANNE E. BERNSTEIN*

PRIMARY sex ratio in mammals is understood to mean the number of males per 100 females at conception rather than at birth, the latter proportion being called the secondary sex ratio. The primary and the secondary sex ratio are quite unlike each other, for during the nine months of pregnancy a great proportion of embryos are reabsorbed or aborted, and the sex ratio of abortions from all causes, with the exception of malformations, is considerably higher than the sex ratio at birth. Therefore much of the variation in the secondary sex ratio in man and other mammals may be explained satisfactorily by variation in the number of abortions.†

In a study of the genetics of sex ratio variation in man the main problem is, therefore, to separate variation in the primary sex ratio from fluctuations in the abortion rate (especially in avoidable abortions as contrasted to malformations). I hope to have come a step closer towards solving this problem by utilizing in my studies only data from the educated and well-to-do, for whom criminally induced and otherwise (medically) preventable abortions are at a minimum.

The theory that poor prenatal environment and a high proportion of criminally induced abortions lower the sex ratio is well founded on the results of many different studies too numerous to mention in detail. Lawrence[9] discusses these studies in his survey of the literature and writes this summary: "By and large a high social or economic status is accompanied by a greater proportion of male births than is a lower status, and rural districts demonstrate a higher masculinity than urban areas. The most widely accepted explanation for these differences is that high social and economic classes and also rural inhabitants have lower reproductive wastage . . . differential rates of reproductive wastage appear to be the most plausible explanation . . . it likewise readily accounts for the higher sex ratio among legitimate births than among illegitimate births . . ." (See also Bernstein.[3])

This author[2] has reported on a relationship between occupations of fathers and the sex ratio of offspring on data secured from WHO'S WHO IN AMERICA. According to the percentage of women in these occupations, I classified them into three groups, *masculine*, *feminine* and *neutral* occupations. With the exception of farming, this method of classification yielded the same groups of *masculine* and *feminine* occupations as were obtained by Terman[14] with a psychological aptitude test. The relation between occupations of fathers and the sex ratio of offspring were as follows: of 5,400 children of men in *masculine* occupations the percentage of male births was 55.6 percent, whereas of 1,200 children of men in *neutral* occu-

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†In 1949, in order to explain the low sex ratio of 106 reported for the collection of aborted foetuses of the Department of Embryology of the Carnegie Institution of Washington by C. Tietze[15] in *Human Biology*, this author pointed out to the editor of *Human Biology* that the Carnegie Collection contained no criminally aborted foetuses and therefore its proportion of malformed foetuses must be larger than in the general population of abortions. The assumption made by this author at the time that the sex ratio for abortions due to malformations is less than 100 as had been observed for stillbirths, was confirmed in 1951 by McKeown and Lowe[11] on a large sample of abortions collected at a British hospital.

pations, the percentage was 50.1 percent and of 1,800 children of men in *feminine* occupations it was only 45.5 percent. It appeared that the more masculine the occupation of the father and probably the more masculine the father himself, the greater the probability of his having male offspring.

The next question to be considered was: Has the degree of femininity of the mother a similar effect on the sex ratio of the offspring, and if so, is the maternal or paternal effect the stronger? The reference book, AMERICAN WOMEN, contains biographical data on age, occupation, marital status, occupation of spouse plus names and birth dates of children for several thousand women prominent in professional, social or political life in the United States. For this study, data on 1,121 women married between 1912 and 1924 whose husbands were still living were selected. (No earlier marriages were included as this author had found[3] that there is a rise in the secondary sex ratio of the upper social strata at the beginning of the 20th century). The 1,121 women thus selected had a total of 2,377 children. To these data were added 27 families with a total of 37 children for whom both parents were known to be Hollywood movie stars. Again, by the frequency of women within them, the various occupations were classified as *masculine* or *feminine* although no *neutral* group was allowed due to the relative meagerness of the data. The majority of women in the *feminine* group were, of course, housewives who, as officers of women's clubs, merited listing in the reference book, but there were also many women writers, artists and grammar school teachers in this group. In the *masculine* group were mostly lawyers, scientists and women who, though mainly housewives, were active in politics and other civic activities. Of 746 children of *masculine* women, the percentage of male children was found to be 56.6 percent but for 1,668 children of *feminine* women it was only 50.2 percent. This difference of 6.4 percent has a standard error of 2.2 percent and is therefore statistically reliable and significant. A slight bias is created by the omission of offspring of widows since Lawrence[10] found a positive association between sex ratio of children and the lifespan of both parents. For this reason, and probably for others, the present percentage of male births cannot be compared directly with those obtained in the WHO'S WHO series.[2]

Rather, the 2,414 children of the present data were allocated to one of four groups according to whether the father or mother were in *masculine* or *feminine* occupations. The resuts of this grouping are listed in Table I.

Besides applying the chi-square test which proved to be significant at the 3 percent level, the significance of the difference between the percentages in Table I was tested on the Mosteller-Tukey probability paper,[13] and it was found that no real difference exists between the percentage of male births for groups 2 and 3, but that the percentages for groups 1 and 4 are significantly different from the middle groups.

Although this author[3] had found that the sex ratio does not decrease with increasing birth order in present-day upper class families as is the case in the general population, it was considered advisable to compute the sex ratio also for "first births only" of the four groups of families shown in Table I. Eight families had to be omitted in this comparison either because birth order was not given or the first birth was a twin birth of a girl and boy. The incidence

TABLE I. Percentage of male offspring for four groups of parents

Group*	No. of families	No. of children	No. of sons	% of sons	Sex ratio
1	278	561	327	58.2 S. E. 2.1	140.0
2	535	1,200	621	51.8 S. E. 1.4	107.3
3	95	185	95	51.4 S. E. 3.7	105.6
4	240	468	216	46.2 S. E. 2.3	85.7
Total	1,148	2,414	1,259	52.2 S. E. 1.0	109.0

*Group 1: Both parents in *masculine* occupations;
Group 2: Father in *masculine* mother in *feminine* occupation;
Group 3: Father in *feminine* mother in *masculine* occupation;
Group 4: Both parents in *feminine* occupations.

of male births for the remaining 1,140 families is given in Table II.

The data of Table II giving the incidence of male births among first born for the four different groups of matings show, as did the data for "all births" of Table I, that this incidence is significantly higher if both parents are in *masculine* occupations and significantly lower if both parents are in *feminine* occupations. Since abortion rates are at a minimum in these data, the differences in the percentage of sons for the four groups of parents must have existed at conception and are therefore, in all probability, genetic in origin. Furthermore, the genes, or set of genes which are responsible for these differences in the proportion of males conceived among groups of parents as classified by the degree of masculinity or femininity of their vocations or avocations, must be identical or connected with the genes or group of genes which control the masculinity or femininity of men and women.*

Theoretical Considerations

An interesting experiment on the effect of nutrition on the sex ratio at birth, independent of variation in the abortion rate, has been reported by a group of physiologists.[6] The theory advanced by them to explain their findings, may have a bearing on the above results. Their experiments were concerned with the sex ratio for crossmatings of rats which had been fed low protein (7.2 percent to 12.0 percent) and high protein (30 percent to 50 percent) diets. When low protein males were crossed with high protein females, the sex ratio for 420 offsprings was 107. When high protein males were crossed with low protein females, the sex ratio was 145. The hypothesis advanced by Hoelzel, *et al.*[6] was that the state of protein metabolism induced in the well nourished parent, affects the germ cells and enhances the survival rate of embryos of the same sex. Hoelzel[7] has suggested to this author that some of her earlier results[3] could also be explained by this theory, i.e., that in strong males a larger proportion of the X chromosome bearing sperms are destroyed than in weak males, and that more male embryos are reabsorbed in strong females than in weak females. With the present findings on the effect of the degree of femininity of the mother on the sex of the offspring, a more purely genetic theory similar to the theory on sex development advanced by Goldschmidt[5] and Bridges[4] might, however, be more applicable.

Several years ago, Goldschmidt[5] working with *Lymantria*, the gypsy moth, pointed out that the degree of maleness and femaleness must vary considerably among various geographic races of this insect since specific crosses between different races of *Lymantria* gave normal offspring of only one sex plus phenotypic intersexes which had the normal chromosomal constitution of the other sex. For instance the cross of what Goldschmidt called "weak" females (equivalent to what I have been calling *masculine* women) with "strong" males (equivalent to what I have been calling *masculine* men) resulted in normal males plus intersexes with the female chromosomal make-up. Bridges[4] showed for *Drosophila melanogaster* that the factors for maleness—which Goldschmidt had assumed to exist—are located throughout the autosomes, and that the factors for femaleness are located along the X chromosomes. Deviations from the two normal ratios of autosomes to sex chromosomes results, as Bridges showed, in *Drosophila* intersexes.

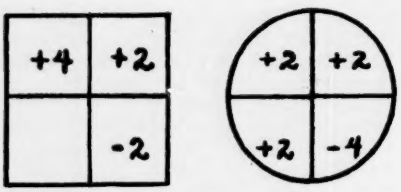
I can explain my present findings on the variation of the human sex ratio according to sex types of the parents by the following theory based on the Goldschmidt-Bridges theory of sex determination and development if I make the additional assumption that intersexes are usually nonviable in mammals. Indeed, clinical cases where the existence of ovitestes in man could be demonstrated are so rare that only 40 such cases have been reported in the entire medical literature. Therefore, I would like to make the following assumption:

The more masculine a man (at present determined by outstanding achievement in a *masculine* profession) the stronger his masculinity genes as located throughout his auto-

TABLE II. Percentage of male offspring among first-born for four groups of parents

Group	No. of families	No. of sons	% of sons	Sex ratio
1	276	163	59.1 S. E. 3.0	144.3
2	532	280	52.6 S. E. 2.2	111.1
3	95	51	53.7 S. E. 5.1	115.9
4	237	109	46.0 S. E. 3.2	85.1
Total (first-born)	1,140	603	52.9 S. E. 1.5	112.3
Total (2nd and later births)	1,274	656	51.5 S. E. 1.4	106.0

*The incidence of male births, as the last two lines of Table II show, is slightly higher for first births than for later births; however, in the present data, this difference is statistically not significant.



"STRONG" MALE AND "WEAK" FEMALE

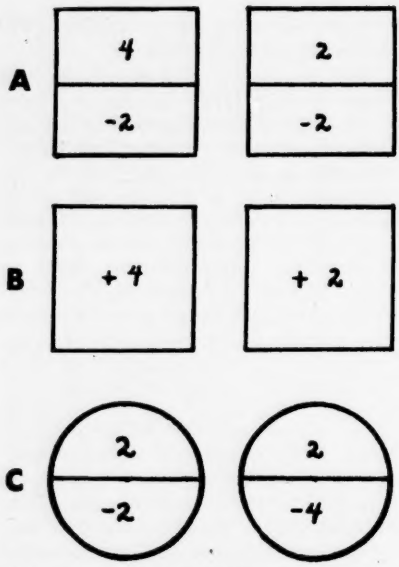
Figure 7

For the male on the left the excess of maleness is +4, where +4 and −4 are the largest values for sum totals of "masculinity" and "femininity" genes. The quadrants on the left represent the genes inherited from the father, and those on the right the genes inherited from the mother. On the right is shown a "weak" female of +2.

somes and the weaker his femininity genes as located on his one X chromosome. Also, the more feminine a woman (similarly determined at present by outstanding achievement in a *feminine* profession) the stronger her femininity genes as located on her two X chromosomes and the weaker her masculinity genes as located on her autosomes. Let us represent the genotype of a strong male as in Figure 7*A* where a square is subdivided by two lines into four quadrants. If masculinity genes are, in accordance with Goldschmidt's system, assigned positive values and femininity genes negative values, then the numbers entered into quadrants one and two represent the sum total of the valences of all the masculinity genes as located on the autosomes, and the negative numbers in quadrants three and four represent the sum total of the valences of the femininity genes on the X chromosomes. In males, of course, the third quadrant is empty since there is only one X chromosome. Thus the two quadrants on the left of the center represent the genes inherited from the father, and the two quadrants on the right represent the genes inherited from the mother. For the man in Figure 7*A* the excess of malenes is +4, and thus a *strong* male in this particular example where +4 and −4 are the largest values for sum totals of *masculinity* and *femininity* genes.

The sperms of the strong male shown in Figure 7*A* are illustrated in Figure 8*A* and *B*, and the eggs of the weak female shown in Figure 7*B* are illustrated in Figure 8*C*. The genotypes of sons and daughters from random mating are shown in Figure 9.

By hypothesis, intersexuality due to genetic conflict are assumed nonviable in man (actually also in *Lymantria* and *D. melanogaster* the intersexual types are much less viable than normal males and females). Thus



MASCULINITY-FEMININITY GENES IN GAMETES

Figure 8

A and *B* represents the X and Y chromosome bearing sperms of the *strong* male shown in Figure 7. *C* represents the eggs of the *weak* female shown in Figure 7.

son No. 4 and daughters No. 2 and No. 3 having neutral valence are nonviable. The same holds true of course, for daughter No. 1 who even has a positive, masculine valence. Therefore, in this particular mating of a strong male and weak female, the sex proportion of three males and one female is a significant deviation from the 1:1 ratio. Of course, the "elimination" of son No. 4 and daughters Nos. 1, 2 and 3 may already have taken place before conception, i.e., it may be that a situation exists similar to that found by Moewus[12] in algae so that no fertilization can take place unless the attraction between certain sex substances called gamones by Moewus and believed to be secreted by egg and sperm into the fluid surrounding them, is definitely positive or negative in sexual valence.

By an application of the above scheme of genetic control of sex ratio in mammals, one can also explain satisfactorily the interesting results obtained by King[8] on the sex ratio of albino rats. After inbreeding rats for six generations in order to obtain purer lines, she selected for strain A rats from litters with a predominance of males and for strain B rats from litters with a predominance of females

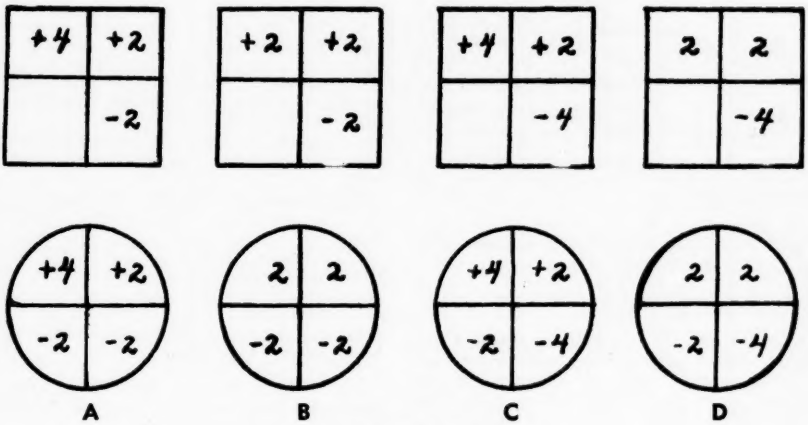


Figure 9

From the gametes shown in Figure 8, eight kinds of progeny are possible, ranging from strongly masculine males (+4) to "intermediates" ("zero" male and +2 female) to strongly feminine female (−2). Male type D and female types A, B and C are nonviable.

After 15 generations of such selected mating, the secondary sex ratio of line A had risen to 125, and in line B it had dropped to 83. In line A selection was probably for male rats with strong masculinity genes, and for female rats with weak femininity genes; in strain B the selection was probably the reverse.

Summary

The author points out that, in order to study variation of the human sex ratio at conception, one has to eliminate as much as possible of the effect of variation in abortion rate in view of the fact that the sex ratio of abortions is much higher than the sex ratio at birth.

In a former study[2] dealing with the sex ratio of offspring of men listed in WHO'S WHO IN AMERICA the author found a strong relationship between occupations of fathers and the sex ratio of offsprings. The more *masculine* the occupation of the fathers (relative low proportion of outstanding women in these occupations) the higher the percentage of sons, and the more *feminine* the occupation of the fathers (relative high proportion of outstanding women) the higher the percentage of daughters.

With data obtained from AMERICAN WOMEN a similar study was made on the effect of the degree of femininity of the occupations or avocations distinguishing the entrants and the sex ratio of the children. For 746 children of women in *masculine* occupations (lawyers, politicians, natural scientists, engineers, physicians, dentists, businessmen and other executives, etc.) the percentage of sons was 56.6 percent whereas for 1,668 children for women in *feminine* occupations (artists, writers, clubwomen, musicians, home-economists, librarians, psychologists, religious and social workers, etc.) the percentage of sons was only 50.2 percent. The difference between these two percentages was 2½ times its standard error, and thus statistically significant.

A finer breakdown into four groups was made by classifying the children according to the occupations of both parents. When both parents were in *masculine* occupations, the percentage of males among 561 children was 58.3 percent, S.E. 2.1; when both parents were in *feminine* occupations, the percentage of males for 268 children was only 46.2 percent, S.E. 2.3; when the father was in a *masculine* and the mother in a *feminine* occupation the percentage of males for 1,200 children was 51.8 percent, S.E. 1.4. This was about equal to 51.4 percent, S.E. 3.7—the percentage of sons for families with a total of 185 children in which the father was in a *feminine* and the mother in a *masculine* occupation. By chi-square test and binomial probability the differences among the three main groups were found to be statistically significant. It was not caused by differences in family size since almost identical percentages were obtained for first births only.

The author believes her findings indicate that Goldschmidt's theory of strong and weak sex types as developed for *Lymantria* may well apply also to humans and other mammals if we stipulate that genetic intersexes are almost never viable in these forms.

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Radiation Effects on Living Systems

(*Continued from page* 58)

radiation work with *Drosophila* and that with maize and other plants, it would have been well to have discussed the work of Stadler on corn.

The ideas set down in this symposium will no doubt be subject to modification as new work comes forth, but this does not detract from the purpose of such a symposium, for it is well to pause at times and take inventory of the present state of affairs in order to better equip ourselves for a more forthright plunge into the new frontiers. The articles in this symposium will serve as a point of reference to the state of our knowledge on radiobiology in 1950.

Even now, just four years since these papers were given, modifications of some of the ideas laid down in the symposium are forthcoming. Recent work involved with the genetic effects of chronic gamma radiation in maize suggests that mutations produced are not directly proportional to dosage. Also there seems to be a threshold around 20 to 40 roentgens per day below which there is no increase of mutation rates over control rates. As new work is done, possible other theories will be subject to change. However, it is well to have the present state of our knowledge so readily available in a single concise volume.

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THE ANATOMY OF THE HETEROCHROMATIN

Data on the Physical Distance Between Breakage Point and Affected Locus in a V-Type Position Effect

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IN a study of variegation at the peach (*pe*) locus in *Drosophila virilis*,[1] an extremely high frequency of mottling was noted in the F_1 individuals resulting from a cross of X-rayed wild-type males to peach females. The *pe* locus resides within the last four bands of basal heterochromatin of chromosome 5 (as seen in salivary gland preparations), and most of the induced breaks were between the last band and the centromere and thus proximal to *pe*. This small salivary heterochromatic region is represented in the metaphase ganglion chromosome 5 by a region measuring at least two-fifths of the chromosome length. Since the condition of the chromosomes in *Drosophila* sperm has been found by Dr. Kenneth Cooper (personal communication) to resemble that of somatic metaphase chromosomes, the high frequency of mottling could be explained by assuming that breaks within this long heterochromatic region are capable of evoking variegation even if they are far from *pe*. Alternatively, the region close to *pe* might be unusually susceptible to X-ray breakage. Evidence will be presented here showing the former explanation to be correct.

It became possible to attack this problem when three translocations causing variegation were obtained, each of which had one of its two breakage points at almost the same location in the euchromatin near the tip of chromosome 3. The breakage points in chromosome 3 of these 3;5 translocations are as follows: pe^{m53} is broken between 3B2a and b, pe^{m51} between 3B2c and d, pe^{m4} between 3B2e and h (see salivary gland chromosome map in Patterson, *et al.*[4]). Since these breaks occur within only seven or less bands of each other, any pronounced difference in length of the translocated chromosomes among the three rearrangements, as observed in ganglion cells, would indicate that chromosome 5 has been broken at different places. From genetic evidence[1] it is known that the break in chromosome 5 in the case of pe^{m4} is proximal to *pe* and it is observed cytologically to have occurred between the last salivary band and the centromere. The break is also observed after the last band in the case of pe^{m51} and pe^{m53} and genetic evidence indicates that the break is proximal to the locus in these rearrangements. Therefore, any difference in length of the translocated ganglion chromosomes among the rearrangements is a minimum measure of the physical distance between *pe* and a point of breakage which can affect the action of this gene.

Cytological preparations of ganglion chromosomes from female larvae bearing the different rearrangements were made and examined. It became immediately obvious from a study of these chromosomes, that the length of the translocated chromosomes varies among the different rearrangements. For example, in pe^{m51} the 5^3 chromosome is very small (Figure 10*A*). Thus the break in the heterochromatin of 5 must have occurred very close to the centromere. The complementary 3^5 chromosome should be very long and should contain a large portion of the basal heterochromatin of 5 located interstitially (Figure 12). In the prometa-

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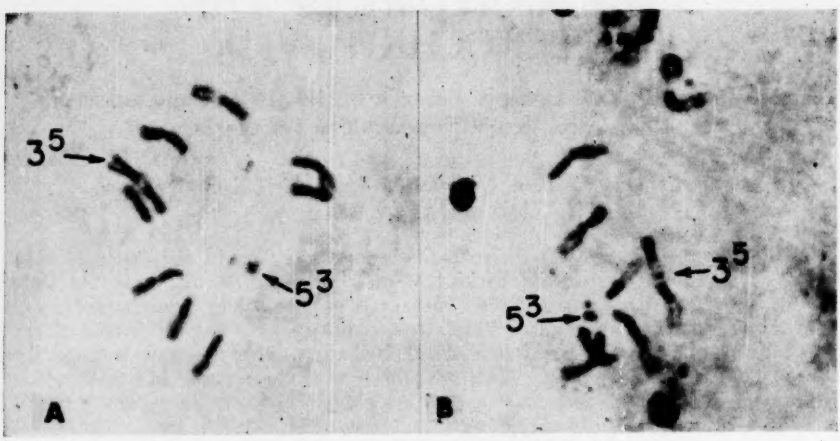


PROMETAPHASE CONFIGURATION

Figure 10

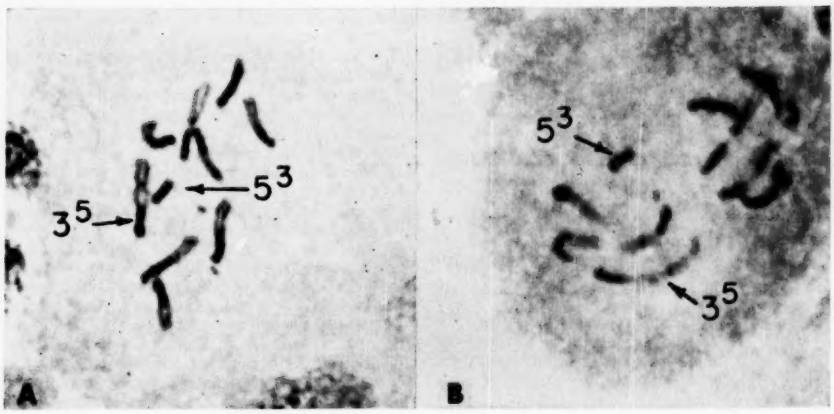
Ganglion chromosomes of $T(3;5)pe^{m51}$. *A* and *B* are from the same larval ganglion.

phase configuration shown in Figure 10*B*, both the basal heterochromatin of 3 and 5 can be clearly seen in the 3^5 chromosome. However, pe^{m53} presents a different cytological picture. In this rearrangement the 5^3 chromosome is relatively long (Figure 11*A*), thus indicating that the heterochromatin of 5 is broken close to *pe* with the result that most of the 5 heterochromatin is located in the 5^3 chromosome. In Figure 11*B* it can be seen that there is no clear evidence of heterochromatin inserted interstitially in the 3^5 chromosome.

In order to obtain quantitative information on the question in point, photomicrographs were made of a series of metaphase configurations, in which all the chromosomes were well spread and lying in one plane, from female larvae heterozygous for a given translocation. Measurements were made with a pair of dividers of the length of all chromosomes (with the exception of the dot-like sixth) as seen in enlargements of the photomicrographs. The lengths of the 3^5 and the 5^3 chromosomes as the percentage of the total length of all rod chromosomes are given in Table I. These measurements indicate that chromosome 5 is broken at different points in the three rearrangements. In the case of pe^{m51}, the break is closest to the centromere, while in pe^{m4} the break is farthest from the centromere and thus closest to *pe*. An intermediate location is found in pe^{m53}. The reliability of these measurements is shown by the fact that in the ten cells measured, the sum of the lengths of the two translocated chromosomes remains reasonably constant. Since chromosomes 3 and 5 are of about equal length, the difference in length of the translocated chromosomes between

TABLE I. Relative length of the 5^3 and 3^5 chromosomes of pe^{m51}, pe^{m53}, and pe^{m4} as measured in metaphase ganglion cells

Rearrangement		Length as percentage of total length of all rod chromosomes		
		5^3	3^5	$5^3 + 3^5$
51		2.3	15.3	17.6
		2.7	16.4	19.1
		2.5	15.7	18.2
	Average	2.5	15.8	18.3
53		4.7	13.9	18.6
		3.5	15.0	18.5
		5.9	14.0	19.9
		4.6	13.8	18.4
	Average	4.7	14.2	18.9
4		6.2	12.8	19.0
		5.0	13.3	18.3
		5.2	13.0	18.2
	Average	5.5	13.0	18.5



LOCATION OF THE 5 HETEROCHROMATIN

Figure 11

Ganglion chromosomes of *T(3;5)pe*^{m53}. *A* and *B* are from different larvae.

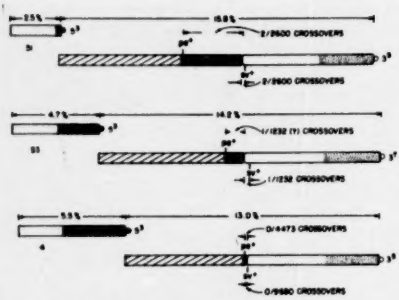
pe^{m51} and *pe*^{m4} indicates that chromosome 5 may be broken at least a third [(3/18.6)×2] of its length away from *pe* and still induce variegation at this locus.

It was hoped that further evidence on the difference in location of the chromosome 5 break in the three rearrangements could be obtained by determining whether the frequency of crossing over between *pe* and the point of breakage varied among the mottles. However, as might be expected, the frequency of crossing over in a heterochromatic region close to a breakage point of a translocation is too low to provide anything but suggestive results. Only two crossover flies were obtained from *pe*^{m51} in 2600 flies examined, one in 1232 from *pe*^{m53}, and none in 4473 from *pe*^{m4}. However, these data do support the cytological picture which shows that the break in *pe*^{m4} is closer to *pe* than it is in the other two rearrangements.

A piece of incidental information of some interest was disclosed in these crossover studies. Not only was the crossover frequency between *pe* and the break obtained, but also the frequency between *sv* and the point of breakage. (*sv* = short veins, a recessive mutant whose locus was reported by Fujii[3] to be at the end of the 3B2 region of chromosome 3.) It was found with *pe*^{m4} that R(*pe*+)/*pe*; *sv*/*sv*+ flies show a typical short-veins effect; i.e., L2, L3, and L4 do not reach the costa of the wing. With the homozygous rearrangement, R(*pe*+)/R(*pe*+); *sv*+/*sv*+ the effect is even more striking than in the homozygous mutant; i.e., L2 is entirely absent in most cases and L3 and L4 stop farther from the costa. This raises the question as to whether this is a position effect of the heterochromatin on the euchromatic locus, *sv*, or whether this locus has been deleted (or inactivated) in the rearrangement. The latter possibility seems more likely for two reasons. In the first place, no crossovers have been observed between *sv* and the break in *pe*^{m4} although 9680 flies have been tested. With each of the other two rearrangements about 0.1 percent crossing over has been found in this region. This evidence is not critical, since the break in chromosome 3 in *pe*^{m4} is closer to *sv* than in the other two rearrangements. In the second place, if this were a position effect, the spreading phenomenon should cause the short-veins phenotype to show up in rearrangements *pe*^{m51} and *pe*^{m53}, but such is not the case. It should be noted that these results verify the location of *sv* reported by Fujii.[3]

The foregoing results are presented graphically in Figure 12. It should be remembered that the distances shown between *pe* and the point of breakage are minimum estimates.

Since the break in chromosome 5 in all three rearrangements is after the last salivary band, it is evident that there is a large block of centromeric heterochromatin in the



LENGTH OF THE METAPHASE CHROMOSOMES

Figure 12

Diagrammatic representation of the relative lengths of the metaphase chromosomes in translocations pe^{m53}, pe^{m58} and pe^{m4}. The euchromatin of chromosome 5 is shaded and heterochromatin cross hatched. The euchromatin of chromosome 3 is shown only in outline while the heterochromatin is stippled.

ganglion chromosome which is not identifiable with any salivary structure unless it be the alpha-heterochromatin (the small darkly staining granular bodies at the base of each chromosome which normally fuse in the chromocenter). This supports the contention of Fujii[3] that the inert regions of the mitotic chromosomes of *D. virilis* are represented in the salivaries by the alpha-heterochromatin.

Discussion and Summary

It is difficult to evaluate the significance of the fact that a heterochromatic break will affect the action of a gene removed from the break by a distance of at least one-third the length of a metaphase chromosome, since the relative distance would be much less in a prophase chromosome where euchromatin is greatly lengthened in comparison with basal heterochromatin. The latter condition would be comparable to the salivary gland chromosomes where measurements have been made of the distance over which the spreading effect in euchromatin occurs. Demerec,[2] using Notch rearrangements, showed that the position effect could spread in the case of $N^{264\text{-}52}$ from the locus of *rst* to at least *bi*, a distance of about one-fifteenth the length of a salivary gland X chromosome.

One of the many fundamental questions about the spreading effect which remains unanswered is the distance between the heterochromatic break and the affected locus *at the time* the suppression of gene action takes place. This distance may be a function only of the distance along the chromosome thread, but, on the other hand, it may depend on the external chromosome length and thus on the stage of the chromosome cycle. Granted that the latter condition is correct, one would be inclined first to think that the resting (metabolic) nucleus would be the period of action. However, it should be recalled that position-effect breaks act only on genes on the same chromosome, genes on the homologue being unaffected. This might suggest that in an organism exhibiting somatic pairing, the suppression takes place at a stage when there is a barrier (space or matrix) separating the homologues. In any event, it is not possible at the present time to state with assurance whether or not the position effect may act over longer distances of heterochromatin than euchromatin.

The one fact which is clearly demonstrated by these data is that breaks in widely separated regions of the basal heterochromatin are effective in evoking variegation at the *pe* locus. This lends support to the hypothesis[1] that the basal heterochromatin functions as a unit and that a disruption of its continuity (by rearrangement) is an important factor in producing V-type position effects.

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INTERSPECIFIC AND INTERVARIETAL HYBRIDIZATION IN SANSEVIERIA

JAMES B. PATE, J. FRANK JOYNER AND EDWARD O. GANGSTAD*

VARIATION in the genus *Sansevieria* is indicated by the number of recognized species. In a monograph of the genus in 1915, Brown[1] described 54 species. In 1941 Greenway[3] noted that eight more species had been described, and pointed out the adaptation and growth in Africa of some species in extremely dry regions, others in wet, tropical forests and some on coral rock. Gangstad *et al.*[2] in 1951 noted differences in leaf, rhizome and root characteristics of seven species. They report that the narrow, petiolate leaf species are best suited for fiber production in South Florida, and within this group of species, *S. trifasciata* Prain is most desirable, because of its tolerance to cold, intermediate percent fiber and good length of leaves. *S. trifasciata* var. *laurentii* (DeWild.) N.E. Br. is reported as having a higher degree of cold tolerance, but lower percent fiber and shorter leaves. *S. liberica* Ger. & Labr. was found to have the highest fiber content of the species studied.

A breeding program for the development of fiber types better adapted to South Florida has been under way since 1951.[5] Interspecific and intervarietal hybridization studies involving *S. trifasciata, S. trifasciata* var. *laurentii, S. thyrsiflora* Thunb. (*S. guineensis* Willd.), *S. parva* N. E. Br., *S. longiflora* Sims, *S. liberica, S. cylindrica* Boj. and *S. deserti* N. E. Br. have been carried out.[4] Hybridization technique, results of hybridization and growth characteristics of hybrids are reported in this paper.

Hybridization Technique

The inflorescence of the species used in these studies is a spike-like raceme with flowering occurring over a period of several days. Flowers open in the late afternoon or early evening with anthesis occurring shortly afterwards. During the period 8-10 a.m., flowers which would normally open later in the same day were emasculated by using forceps to open the flower and remove the anthers. The following morning emasculated flowers were pollinated by touching the stigmas with pollen-shedding anthers from the male parent. All pollinations were made in the greenhouse, and the inflorescence of male and female parents was covered with Kraft bags except during emasculation and pollination.

Results of Hybridization

S. trifasciata is compatible wtih S. *trifasciata* var. *laurentii, S. parva, S. liberica* and *S. deserti* (Table I). Hybrids of *S. trifasciata* var. *laurentii* with *S. parva* and *S. parva* with *S. deserti* were also obtained. The percent seed set in all compatible crosses is low. Hybrids of *S. trifasciata* with *S. liberica* are very difficult to obtain.

Mature fruit developed in crosses of *S. trifasciata* × *S. cylindrica,* but no seed were produced. There was no material stimulation in the reciprocal cross.

S. longiflora and *S. cylindrica* produce a number of seed under South Florida conditions, but no interspecific hybrids involving these two species were ob-

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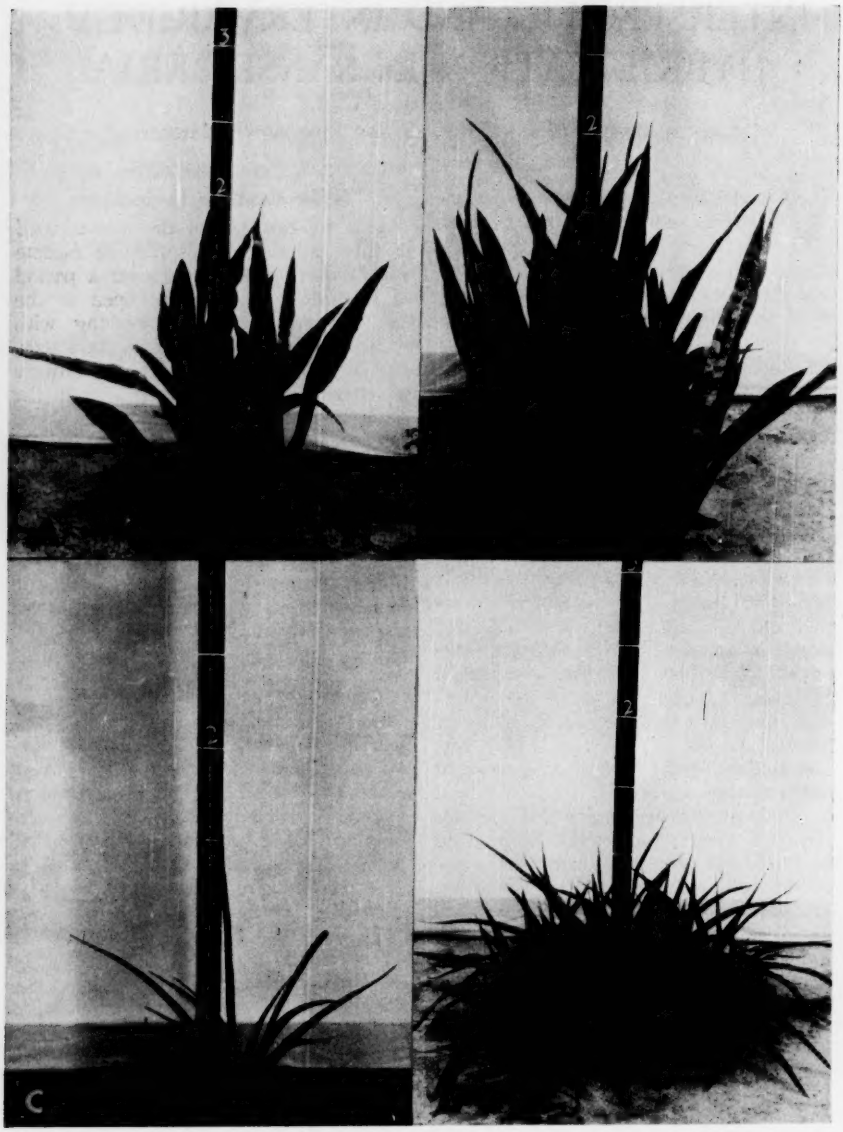
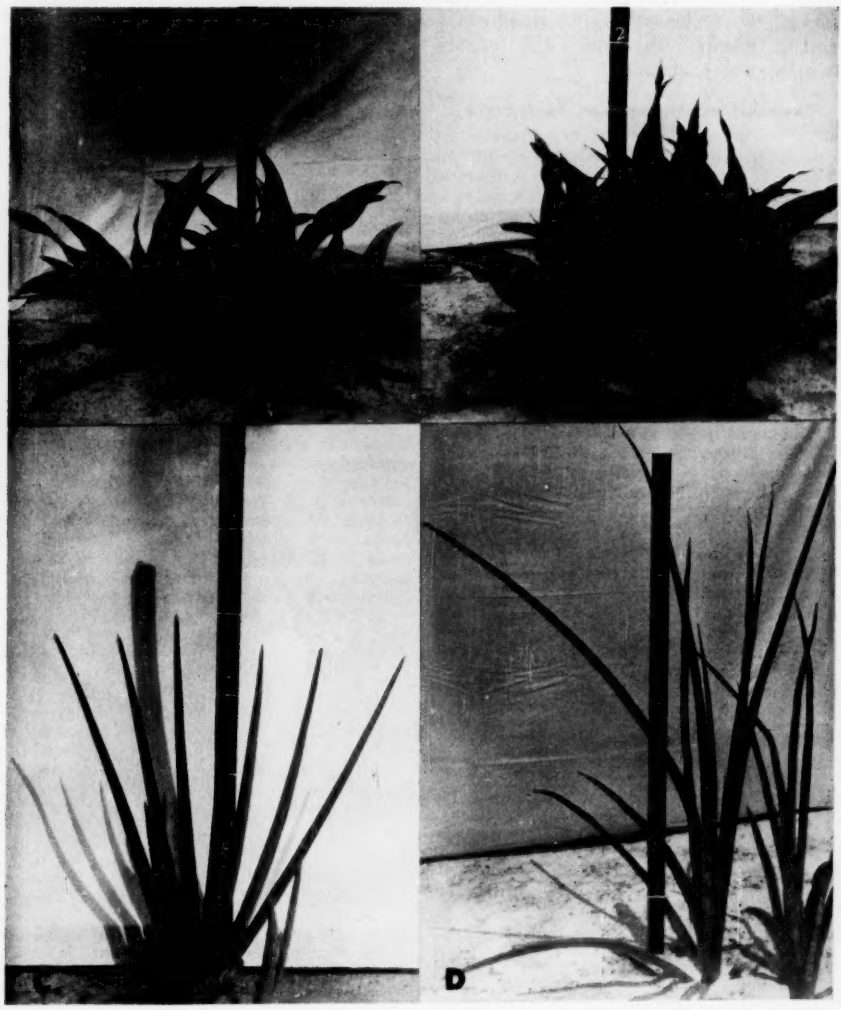


HYBRIDS OF *SANSEVIERIA TRIFASCIATA* WITH PETIOLATE LEAF SPECIES

Figure 13

A — *S. trifasciata*; *B* — *S. trifasciata* × *S. trifasciata* var. *laurentii*; *C* — *S. parva*; *D* — *S. trifasciata* × *S. parva*. *A*, *B* and *D* are seedling clones at the end of the second year's growth, and *C* is a single plant from a two year stand.



HYBRIDS OF *SANSEVIERIA TRIFASCIATA* WITH A BROAD AND A ROUND LEAF SPECIES

Figure 14

A — *S. liberica*; *B* — *S. trifasciata* × *S. liberica*; *C* — *S. deserti*; *D* — *S. trifasciata* × *S. deserti*. *A*, *B* and *D* are seedling clones at the end of the second year's growth, and *C* is a single plant from a two year stand.

tained. *S. thyrsiflora* has not been observed to produce seed in South Florida, and no hybrids were obtained in crosses involving this species.

Growth Characteristics of *Sansevieria* Hybrids

Seed of crosses of *S. trifasciata* with *S. trifasciata* var. *laurentii, S. parva, S. liberica* and *S. deserti* were obtained simultaneously in 1951 and were planted in four-inch pots in the greenhouse. Seed of the common parent, *S. trifasciata,* were also planted. Seedling plants were transplanted to the field near Lake Worth 15 months later.

Data on number of plants per seedlings and length of longest leaf were taken after one and two years' growth (Table II). Differences between groups of seedlings in number of plants were significant after both one and two years' growth. The largest number of plants was obtained in the *S. trifasciata* × *S. parva* hybrids. Differences in length of leaf during the second year of growth were highly significant. Longest leaf length was obtained in the *S. trifasciata* × *S. deserti* hybrids.

Pictures of hybrids and parents are shown in Figures 13 and 14. Hybrids of *S. trifasciata* × *S. trifasciata* var. *laurentii* are very similar to seedlings obtained from *S. trifasciata.* The *S. trifasciata* × *S. parva* hybrids are characterized by short, narrow petiolate leaves with long subulate points. Their growth in height does not differ greatly from the *S. parva* parent. The *S. trifasciata* × *S. liberica* hybrids have a broad flat leaf with a definite reddish-brown fiberous margin and short subulate point and are similar to *S. liberica.* Hybrids of *S. trifasciata* × *S. deserti* do not particularly resemble either parent. The leaf is semi-round with an interfacial channel extending the full length of the leaf. The length of the leaf is greater than either parent.

The most desirable natural species, *S. trifasciata,* has been combined with *S. trifasciata* var. *laurentii,* the type most tolerant to cold; with *S. parva,* a prolific plant producing species and with *S. liberica,* a species having a high percent fiber. The hybrid of *S. trifasciata* × *S. deserti* appears to have considerable hybrid vigor.

TABLE I. Percent seed set in crosses of *Sansevieria*

Cross	No. Flowers Pollinated	% Seed Set
S. trifasciata × *S. trifasciata* var. *laurentii*	1765	16.08
S. trifasciata var. *laurentii* × *S. trifasciata*	815	19.38
S. trifasciata × *S. thyrsiflora*	175	0
S. trifasciata var. *laurentii* × *S. thyrsiflora*	204	0
S. thyrsiflora × *S. trifasciata*	109	0
S. trifasciata × *S. parva*	166	4.23
S. trifasciata var. *laurentii* × *S. parva*	54	12.96
S. trifasciata × *S. longiflora*	129	0
S. longiflora × *S. trifasciata*	280	0
S. trifasciata × *S. liberica*	1416	.42
S. trifasciata var. *laurentii* × *S. liberica*	161	0
S. liberica × *S. trifasciata*	165	0
S. trifasciata × *cylindrica*	382	0*
S. cylindrica × *S. trifasciata*	142	0
S. trifasciata × *S. deserti*	223	16.59
S. thyrsiflora × *S. parva*	72	0
S. thyrsiflora × *S. longiflora*	112	0
S. longiflora × *S. thyrsiflora*	76	0
S. thyrsiflora × *S. cylindrica*	117	0
S. thyrsiflora × *S. deserti*	240	0
S. parva × *S. deserti*	49	7.65
S. liberica × *S. longiflora*	177	0
S. cylindrica × *S. deserti*	64	0

* Parthenocarpic fruit produced.

Summary

Twenty-three crosses, involving *S. trifasciata, S. trifasciata* var. *laurentii, S. thyrsiflora, S. parva, S. longiflora, S. liberica, S. cylindrica* and *S. deserti* were made. All combinations were not possible because of species differences in season of flowering. Hybrids were obtained in four interspecific crosses: *S. trifasciata* × *S. parva* (also *S. trifasciata* var. *laurentii* × *S. parva*), *S. trifasciata* × *S. liberica, S. trifasciata* × *S. deserti* and *S. parva* × *S. deserti* and in the intervarietal cross of *S. trifasciata* × *S. trifasciata* var. *laurentii.* Maternal stimulation occurred in the cross of *S. trifasciata* × *S. cylindrica.*

Hybrids of *S. trifasciata* with *S. trifasciata* var. *laurentii, S. parva, S. liberica* and *S. deserti* were compared with the common parent species, *S. trifasciata.* Significant differences were noted in number of plants and leaf length. Extreme vigor for length of leaf was observed in the *S. trifasciata* × *S. deserti* hybrids.

The importance of the hybrids in the development of a variety for fiber production in South Florida is emphasized.

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TABLE II. Means and standard errors of number of plants and length of longest leaf in *Sansevieria* hybrid and parent seedlings after one and two years' growth

Parent or Hybrid	No. of Seedlings	1 Year's Growth: No. of Plants	1 Year's Growth: Length Longest Leaf, in.	2 Years' Growth: No. of Plants	2 Years' Growth: Length Longest Leaf, in.
S. trifasciata	13	2.3±.2	5.5±.2	4.7±.8	15.8±4.1
S. trifasciata × *S. trifasciata* var. *laurentii*	15	2.3±.2	5.2±.4	11.6±.9	19.3±1.1
S. trifasciata × *S. parva*	7	3.7±.4	5.2±.4	14.6±1.2	19.4±.8
S. trifasciata × *S. liberica*	2	1.5±.5	3.2±.5	8.0±2.0	21.0±3.0
S. trifasciata × *S. deserti*	6	1.0±.0	5.5±.7	5.1±1.0	43.0±3.3

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SINUS ARYTHMIA, A HEREDITARY CHARACTERISTIC

In the House Mouse

CLYDE KEELER AND AMELIA VICARI*

SEVERAL years ago in an attempt to study the brain waves of mice, we applied the electrodes of a Garceau Junior Electroencephalograph to a number of adult animals belonging to different strains which were kindly made available by the Jackson Memorial Laboratory. As a routine procedure we immobilized the mice on their backs and took breathing records by placing needle electrodes in the skin at the sides of the chest. Heart rate records were obtained by placing one electrode in the skin above the lower portion of the sternum and the other in the skin of the left side of the chest.

With one exception, ventricular beats in our series of mice were quite regular in their timing as shown by our electrocardiograms thus taken. Normal heart beats occurred with a frequency of 630 per minute in C.E. ♂ 213 (Figure 15, record No. 10) and 636 per minute in N ♀ 221 (Figure 15, record No. 11). These will serve as controls with which to compare other records.

The exceptional mouse of this series was T⁶♀2184 E/E from Dr. Snell's translocation stock. The record for this mouse is shown in Figure 15, No. 1. The reader will note a series of 13 well-timed beats, a skip, 19 beats, a skip, seven beats, and a skip. This irregularity is shown throughout a long record that we obtained on two different days.

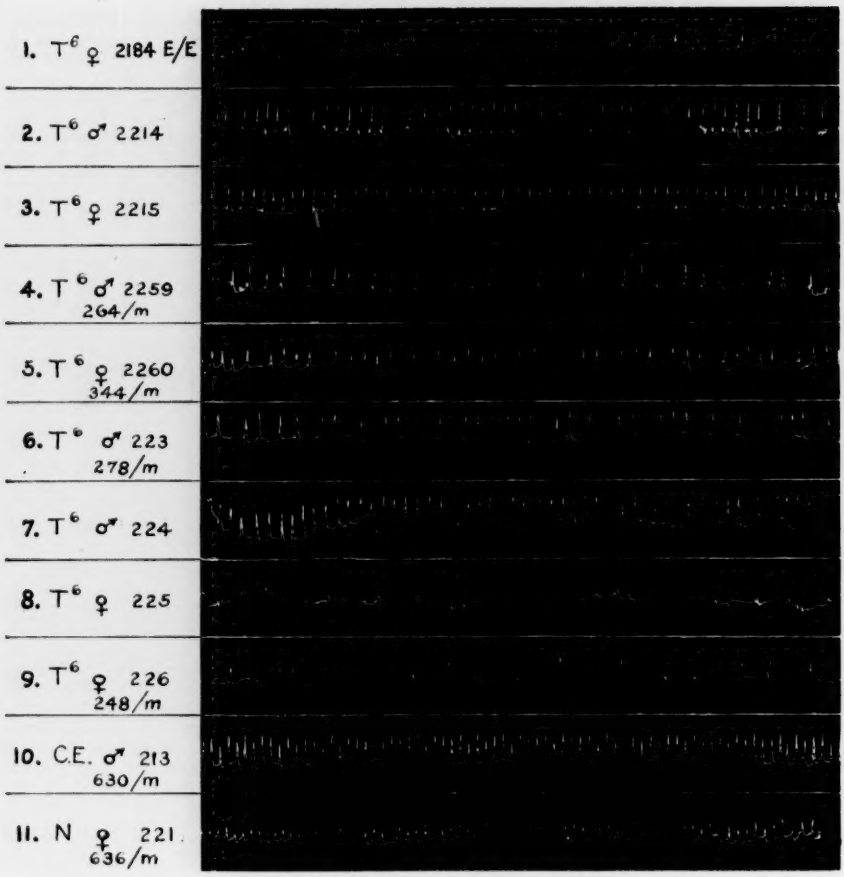
When the geneticist encounters such unusual function he immediately wonders about the possibility of heredity and Dr. Snell kindly provided close relatives of the abnormal mouse for testing.

We obtained records from T⁶♂2214, T⁶♀2215, T⁶♂2259, and T⁶♀2260 which are siblings of the arythmic mouse. Their cardiograms are shown in Figure 15, Nos. 2, 3, 4 and 5 respectively. Slight irregularities are noted in the records of T⁶♀2215, and gross irregularities in the records of the other three siblings with the number of beats per minute reduced considerably below normal. T⁶♂2259 registered 264 beats per minute. T⁶♀2260 shows a slightly faster rate of 344 beats per minute.

T⁶♂2259 had been mated to T⁶♀2260 and four of their young were available for testing. Their records are shown in Figure 15, Nos. 6, 7, 8, and 9. T⁶♂223 has about as many heart beats per minute (278/m) as his father, the slowest parent. T⁶♂224 is almost normal T⁶♀225 has a weak record but it shows definite sinus arythmia. T⁶♀226 shows irregularities in timing, and a slow rate (248/m) like that of her father.

Because the first mouse discovered to have sinus arythmia was a shaker-2 animal, we wondered if the abnormal timing of the heart might be pleiotropically associated with that nervous abnormality. T⁶♂2259, T⁶♂224, T⁶♀225, and T⁶♀226 are all shakers. Our question was answered because T⁶♂224 is a shaker with almost normal record for heart beat.

*Georgia State College for Women and Jackson Memorial Laboratory, respectively.



CARDIOGRAMS OF ARYTHMIC MICE

Figure 15

Record Nos. 2, 3, 4 and 5 are siblings of No. 1, an arythmic mouse. Slight irregularities are seen in No. 3, and gross irregularities in the others. Nos. 6, 7, 8 and 9 are offspring of Nos. 4 and 5. Nos. 10 and 11 are normal mice, used as controls.

whereas T⁶♀2260 and T⁶♂2214 are not shakers and still show marked sinus arythmia.

At this point it is impossible to say what the physical abnormality is which underlies the physiological defect. It might conceivably be due to over-stimulation of the vagus nerve at the vagus centers in the medulla of the brain, or it might be due to a defect in the sinus node of the heart. An examination of the records of the arythmic animals shows that a small auricular beat precedes the ventricular in the normal fashion, and hence we may suggest that once the sinus node has functioned, causing the muscles of the auricles to contract, the pulling of the auricular muscles stimulate the auriculo-ventricular node and it in turn stimulates the auriculo-ventricular bundle that sets off the contraction of the ventricles in a normal manner.

It was felt that these observations should be put on record at this time and it is hoped that the study of this sinus arythmia can be continued in the near future.

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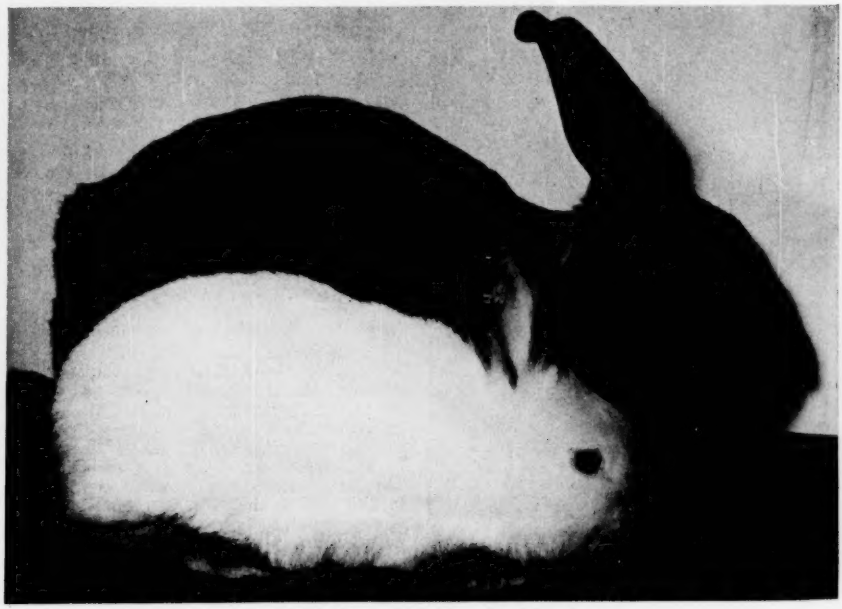
THE AERIAL TRANSPORT OF FERTILIZED MAMMALIAN OVA

M. C. Chang and W. G. R. Makden*



LONG-RANGE FOSTER MATERNITY

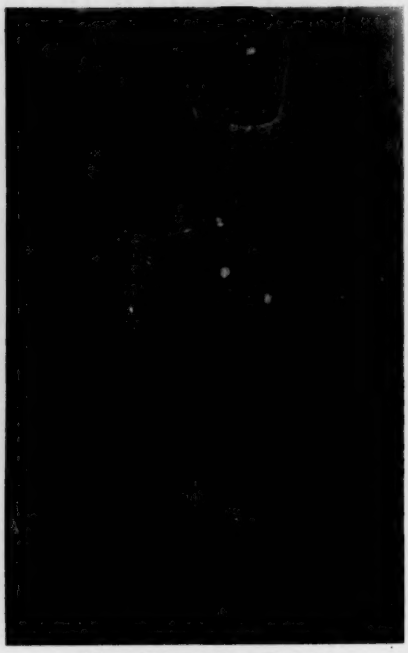
Figure 16

A black foster mother is shown with her Californian young, developed from ova fertilized in the U. S. A. and sent *in vitro* by rail and air to Cambridge, England, for transplantation. Trans-Atlantic foster-maternity of fertilized eggs has been successfully accomplished a number of times.

THE transplantation of fertilized rabbit ova is not new; Heape[7, 8] used the technique to demonstrate that the genetic characteristics of mammals were fixed at conception and that they were not influenced by the interuterine environment of a foster mother. Pincus[10] employed the technique during investigations on fertility and discovered that it was possible to successfully transplant ova to a pseudo-pregnant doe. Chang[3] has been able to store *in vitro* at 10° C fertilized rabbit ova in the early stages and in the late blastocyst stage.

The practical application of such technique in the past seemed remote due to the low percentage of success achieved by early workers. In recent years much more promising results have been obtained. Chang[2] recovered 53 2-cell ova from the superovulation of a single donor doe, which he transplanted into four recipients from which 45 normal young were obtained, or 85 percent survival; Dowling[4] obtained 78 percent,

*Worcester Foundation, Shrewsburg, Massachusetts and School of Agriculture, University of Cambridge, respectively. The authors are indebted to Drs. J. Hammond and G. Pincus, and to Trans-World Airlines for providing facilities for these experiments.



TWO-CELL BABY BUGGY

Figure 17

1 ml. capacity phial used for the aerial shipment of fertilized ova for transplantation.

and Avis[1] 81 percent. This increase in the number of transplanted ova developing into viable young has aroused considerable interest as to its possible practical application to agriculture and medicine. If results equivalent to those already obtained in the rabbit can be achieved in cattle the practical significance will be enormous.

Käppeli[9] has estimated that the ovaries of the three-months-old heifer contain from 75,000 to 297,000 oocytes, while Hammond[6] has pointed out that under modern methods of farming only some four calves are born during a cow's lifetime with the maximum ever achieved being about 20. The hormone control of multiple ovulations in cattle have largely been achieved,[4, 5] and the development of transferred bovine ovum was reported.[12] When techniques become available for the successful transplantation of bovine ova, not only will it permit large numbers of high quality animals to be obtained from a single donor animal, but it will offer a simple cheap method for the exportation of cattle by air to a distant part of the globe; a single glass phial of 1 ml. capacity being capable of containing several thousand fertilized bovine ova.

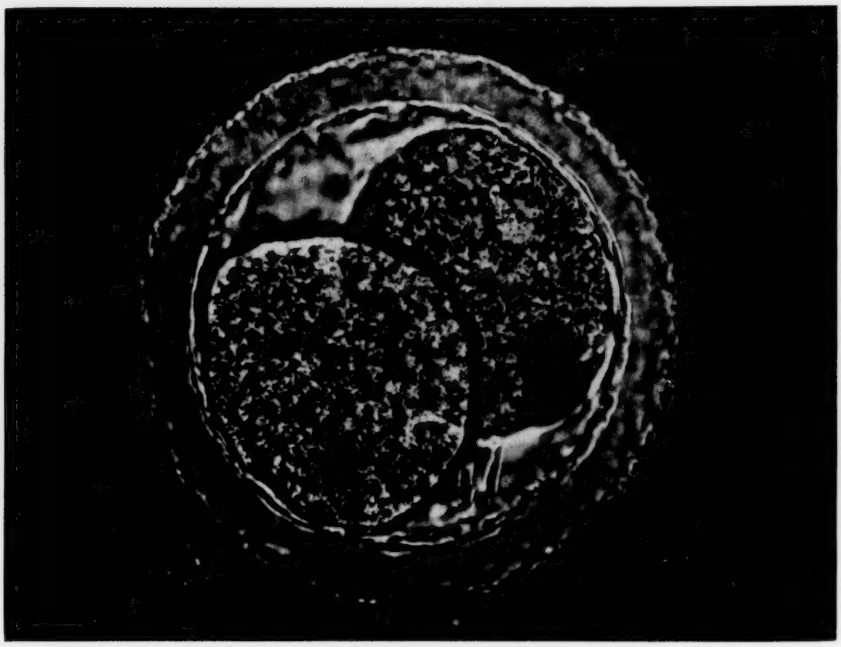
The scope of these experiments was to determine (a) if the fertilized mammalian ova could successfully withstand aerial transport over long distances, and (b) the most suitable medium and stage of development for their survival, as well as the optimum temperature during transport.

Methods and Results

Two shipments have been made from the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, to the School of Agriculture, University of Cambridge, England.

Two Californian rabbits were superovulated by repeated injections of pituitary extract,[11] and inseminated with semen from a Californian buck at the Worcester Foundation for Experimental Biology. About 24 hours after the insemination the does were sacrificed and the Fallopian tubes flushed with whole rabbit serum. In all, 74 fertilized ova at the 2-cell stage were recovered and placed in four small phials (1 ml. capacity) containing whole serum with a drop of penicillin solution added (Figure 17). The phials were then placed in a small thermos flask containing two small ice balloons. The thermos was shipped on November 5, 1951 from Boston to London by air, and thence by bus and train to Cambridge. The time interval from recovery of the ova to the time of transplantation was 27 hours, and the temperature of the thermos was 12°C at the time of packing and 16°C at the time of unpacking.

Three black does kept at the School of Agriculture, University of Cambridge, were intravenously injected with P.U. and two of them successfully mated to a colored vasectomized buck to induce ovluation about 26 hours before the transplantation was made. A midline incision was made on the recipients and five 2-celled ova (Figure 18) placed in the ampulla of each Fallopian tube. Examination of the ovaries, however, revealed that one of the recipients had failed to ovulate. On the 32nd day after the transplantation, two white Californian young were produced by one recipient doe. One of these subsequently died, but the other has grown into a strong healthy rabbit (Figure 16). The percentage of normal development in the two cases where ovulation



TWO-CELLED OVUM

Figure 18

One of the 2-celled ova received in Cambridge from the first shipment for transplantation from the U. S. A.

had occurred is rather low, 10 percent, as compared with those stored by Chang[3] at 10°C (37 percent).

Two albino does were superovulated and inseminated by semen of an albino buck. Twenty-four to 26 hours after insemination these does were sacrificed and the ova washed from the Fallopian tubes with whole serum. Twenty-one 2-cell and 39 4-cell ova were recovered. These were transferred to 4 small phials, packed with an ice balloon, and on January 22, 1952 were shipped in a thermos container by air to London. The time interval between the recovery of the ova in the United States and their transplantation at Cambridge was 30 hours, and the temperature at the time of unpacking was 16°C.

Three black does at Cambridge were used as recipients. They were intravenously injected with 100 i.u. P.U. and mated to a colored vasectomized buck 29 hours before transplantation was made. Each recipient doe received 10 fertilized ova, five being placed in the ampulla of each Fallopian tube. Recipient No. 1 received only 2-cell ova, while Nos. 2 and 3 received ova in the 4-cell stage of development. Normal ovulation had occurred in all three recipients.

Recipient doe No. 2 died of a gastric disorder 28 days after transplantation, and on examination was found to contain 7 fully developed embryos (Figure 19). The third recipient doe gave birth to two normal albino young on the 30th day after transplantation. The percentage development of egg to young from the second experiment was 30 percent as compared with 10 percent in the first experiment. This approaches the percentage of development obtained by Chang[3] for ova stored at 10°C for 24 hours (37 percent). It is of interest that all nine ova which developed into young from the second experiment were shipped in the 4-cell stage, and that 70 percent development occurred in one recipient. This was in spite of the ova remaining in transit for a longer period during the second experiment than the first. Whether 4-cell ova are more resistant to aerial transportation, or whether the increase in percentage of development was due to less variations in temperature at the time of shipment remains for further investigation.

TRANSPLANTATIONAL TRAGEDY

Figure 19

Doe No. 2 was implanted with 10 2-celled ova from America. Four weeks later she died. Her foster-progeny of seven died with her, at an advanced stage of normal development.

Discussion

Although only the results of two preliminary experiments have as yet been completed, these indicate that fertilized rabbit ova can survive air and rail transportation over long distances. In fact, in these initial experiments two different breeds of rabbits were established in England from ova fertilized in the United States. The viability of the ova does not appear to be affected by air or rail transport, the percentage of ova developing after continuous travelling for 30 hours being approximately equal to that obtained by Chang[3] for ova stored at the same stage of development for 24 hours at 10°C. In one recipient seven out of 10 ova transplanted developed; this is comparable with the results obtained from ova directly transplanted to recipients immediately after recovery. The fact that all nine ova developing from the second shipment were shipped in the 4-cell stage may or may not be significant.

Much more work must be done to determine the optimum stage and temperature most suitable for the shipment of fertilized mammalian ova before serious thought can be given to its practical application in agriculture.

Summary

1. Fertilized segmenting rabbit ova are capable of surviving aerial transport over long distances. Two different breeds of rabbits have been established from ova fertilized in the United States and flown *in vitro* to Cambridge University, England.

2. The percentage of transplanted eggs which developed to young was 10 percent in the first experiment, where only 2-cell ova were used, and 30 percent in the second experiment in which all ova developing were shipped in the 4-cell stage.

The ova were shipped in small phials (1 ml. capacity) containing whole blood serum, to which a drop of penicillin had been added. These were put in thermos flasks containing small ice balloons and shipped by air and rail. The time interval from recovery of the ova to time of transplantation was 27 to 30 hours.

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SPERMATOGENESIS IN THE DOMESTIC FOWL

EARL H. NEWCOMER AND JOHN W. A. BRANT*

THERE have been two previous studies of spermatogenesis in the fowl, the first by Guyer[5, 6] and the other by Miller.[19] A number of other studies have been made upon the somatic and occasionally the meiotic metaphase chromosomes, largely in the attempt to achieve a chromosome count or to identify the sex chromosome. The literature on this work has been recently reviewed and summarized by Brant.[2] † The status of these investigations and some of the problems of avian cytology have also been evaluated by Painter and Cole,[24] as follows:

> How is it possible for experienced investigators, studying well preserved material, to reach such diverse conclusions about the diploid number and the identity of the sex chromosome in poultry? . . . On the basis of our experience, as well as a survey of the pertinent literature, it is clear that bird cytology presents difficulties not commonly met with in other animals. . . . The cells of birds are very small, the diploid number is invariably high, sixty to eighty-five elements being reported in recent papers, and the complexes consist of a graded series of small V's, J's, blunt rods and dots. In addition to the natural difficulties of making counts of these small elements in a crowded equatorial plate there are three features which often make enumeration a matter of interpretation: (1) The prophase split often shows prominently at metaphase. This is easy to recognize in large elements but when two dot-like bodies lie closely appressed, should they be considered as two chromatids, or two separate chromosomes? (2) The primary constriction is often so deeply incised that the two arms of a V- or J-shaped chromosome may appear as two separate elements. The failure to recognize this condition has, we think, been the main reason why sex chromosomes have been erroneously identified in poultry and in pigeons. As regards counting, when very small V's or J's are involved it is often impossible to be sure whether one is dealing with two separate chromosomes, the inner ends of which closely abut, or a true V the two arms of which are separated by the point of spindle fiber attachment. (3) Another enigmatic feature is the presence of chromatic threads which often connect two or more bodies together. How are such complexes to be interpreted? Are we dealing with satellited chromosomes, or two separate elements?

In general, the earlier investigators reported the lowest diploid numbers (Guyer, $2n = 18$) and the later investigators have gradually increased the number, culminating in Yamashina[35] who reported a diploid number of precisely 77 in females and 78 in the males of all 17 races of poultry investigated by him. The tendency of contemporary investigators is to accept the highest figure as being probably the most ac-

*Department of Botany, University of Connecticut, Storrs, Conn., and Agricultural Officer, FAO Technical Mission, Department of Agriculture, Paradeniya, Ceylon, respectively. The authors wish to acknowledge the very material assistance of Dr. James R. Carson of the Poultry Department and Dr. Walter Landauer of the Genetics Department of the University of Connecticut throughout the course of this study. Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

†At the time Brant was writing his paper, our preliminary unpublished findings indicated a chromosome number of 17II ± 2 for the fowl. Later work as herein reported shows this to be incorrect.

curate. Thus Miller[19] expressed the opinion:

> Since 1930 the increase in number of small chromosomes has raised prior counts. Obviously these changes are due to improvements of technique and accuracy in observation.

If these observations are correct, the prospect of obtaining sufficient data for locating gene markers on all the chromosomes is indeed dim. Our study, however, does not support the trend to higher numbers, nor, we think, does a perusal of the literature with reference to the techniques used. Matthey[16, 17] has also voiced similar reservations and is of the opinion that with regard to the chromosomal apparatus, the *Aves* are the least known of all the vertebrates.

Genetic evidence has demonstrated the heterozygosity of the female for sex, but the identity of the sex chromosome is still in doubt. Most earlier investigators, following Guyer[6] and Hance,[9, 10] regarded the largest chromosome as associated with sex and the type of chromosome constitution as being XO or XY. Later investigators following Suzuki,[30] Sokolow and Trofimow[29] have chosen the fifth largest, a metacentric V chromosome. Miller's work,[19] based largely upon spermatogenesis in a sex-reversed female, corroborated the findings for the fifth chromosome and seems to constitute the strongest evidence for the identification of the sex chromosome. The consensus of opinion now is that the female is heterogametic for sex and the type is XO for the female and XX for the male. However, Matthey[16, 17] maintains that morphologically differentiated heterochromosomes do not exist in the *Aves*.

There is a substantial amount of genetic information on the domestic fowl,[13] but only a beginning has been made upon the cytological interpretation of these data. Since the fowl is a widely bred animal which offers considerable material for gene mapping, and since the number of linkage groups is as large as the haploid chromosome number, an accurate figure for the latter would give some idea of the magnitude of the job. Furthermore, since the chromosomes in the prophase stages of the primary sex cells reflect their homology or genetic equivalence by their behavior during germ cell formation, comparative studies of the spermatogenesis of the domestic fowl with its various putative progenitors and hybrids between them might throw some light on its origin and mode of evolution. Such studies might also provide an explanation for its sometimes aberrant genetic behavior, and more intensive detailed studies may yield data relevant to the problems of racial origins, stability and sterility.

This study is limited to the development of an adequate technique for the handling of fowl chromosomes, a survey of the details of spermatogenesis, and an effort to determine the chromosome number of one breed of the domestic fowl.

Materials and Methods

Preliminary collections and examinations of testicular tissues of Rhode Island Reds, Barred Rocks and hybrids between them from one to 17 weeks of age were made at weekly intervals for a study of sequential development, time of maturation, and to ascertain the best time for the study of spermatogenesis. With regard to the latter, collections from young and mature birds were made at two-hour intervals throughout a day and night to determine whether there was any periodicity of cell division such as reported by Riley[26] for the sparrow and assumed by Miller.[19] No periodicity was found in any case.*

A study of spermatogenesis of the above groups of fowls revealed a high incidence of meiotic abnormalities which the authors interpreted as due to structural heterozygosity. Since these findings were at variance with those of previous investigators and suggested to some a technical inadequacy, the study was

*Since going to press a paper by J. C. Cross (Oxygen tension as a factor in meiosis *Cytologia* 18:293-295, 1953) has appeared supporting Miller's[19] assumption that meiosis in the fowl normally occurs at night. Of the 50 or more fowls involved in our studies and kept under normal conditions, meiotic divisions were abundant throughout the day. The single fowl used in this study was sacrificed shortly after noon.

repeated using only Rhode Island Red fowl of known origin. The results of this study constitute the present report. Our earlier work clearly indicates the need for further investigation of breed hybrids which may shed some light on breed origins.

Much of the time and effort of our preliminary studies was directed toward securing a smear technique which would present the entire chromosome complex of an intact cell. Adequate staining was at first achieved by modifying the dye solvents,[20] but it was later discovered that the real obstacle in the technique was the Carnoy killing fluid employed. Tissues stored in Carnoy became progressively more difficult to stain and lost their ability to react with Feulgen after several weeks' storage. A new killing fluid was then sought for and found which solved this problem and is now used exclusively. The formula for the new killing fluid by volume is as follows:

6 parts iso-propyl alcohol
3 parts propionic acid
1 part dioxane
1 part petroleum ether
1 part acetone

Both plant and animal tissues have now been stored in this solution for more than a year with no perceptible loss in staining ability with propionic carmine* or Feulgen.[21] For the paraffin-sectioning technique, tissues are removed from this fluid directly into absolute ethanol, followed by toluene or xylene and embedded in paraffin, thus saving the time required for washing and dehydration.

For comparative purposes, testes were also fixed in Bouin's, Flemming's (weak) Hermann's, and our iso-propyl mixture, and subsequently sectioned and stained. The stains used were the haematoxylin, light green combination recommended by Yamashina, the Randolph iodine-crystal violet technique and Feulgen's stain for desoxyribose-nucleic acid. Best results with the paraffin technique were obtained after fixation in the iso-propyl mixture,[21] sectioning at 7-10μ and staining with crystal violet or Feulgen. No sectioning technique, however, compared in general usefulness with the smear technique.

Observations

Prophase stages of primary spermatocytes. The chromosomes of the domestic fowl occur in two size categories, six pairs of relatively large (3.0-6.5μ) chromosomes sometimes called macro-chromosomes and an indefinite number of smaller chromosomes, the micro-chromosomes, grading in size from about 3μ to barely visible granules, all of which are Feulgen positive. Only Yamashina[35] claims a constant number and morphology for the micro-chromosomes, a claim which is not supported by the present study nor by our study of somatic chromosomes (unpublished).

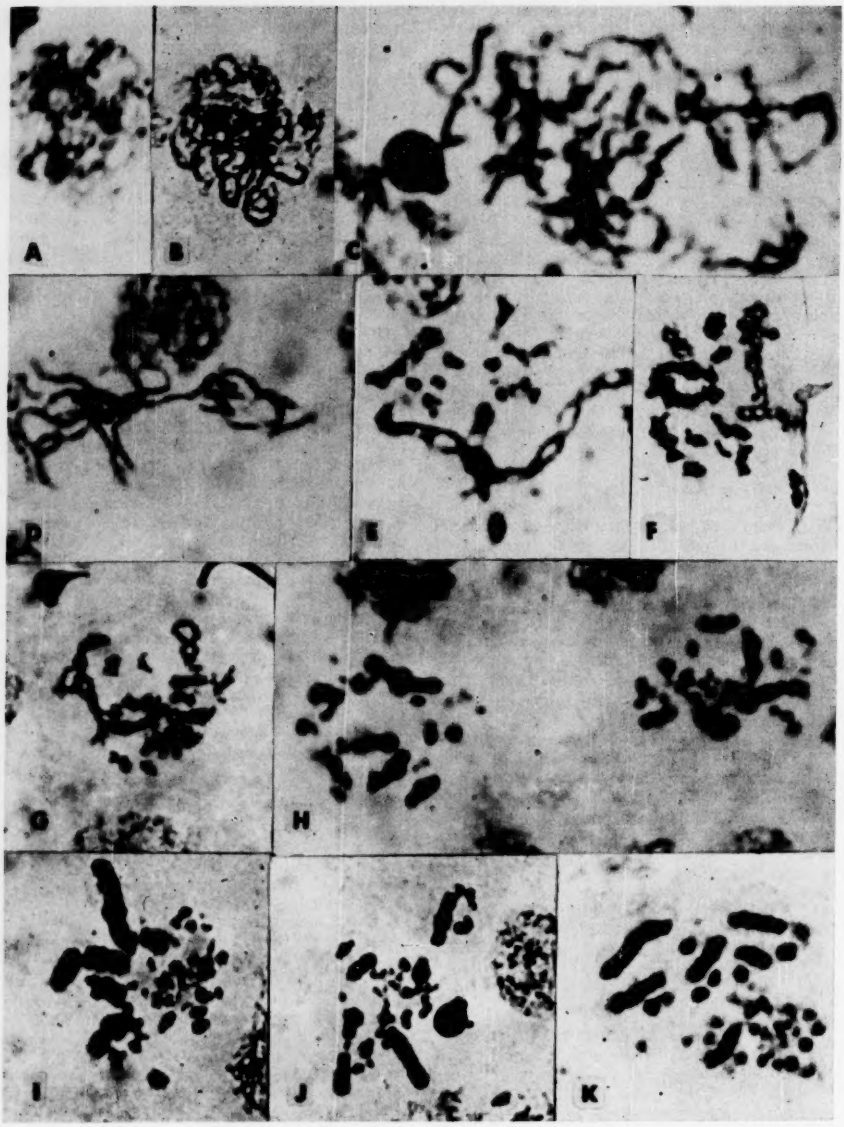
In the pre-prophase primary spermatocyte (Figure 20*A*), heterochromatic masses are conspicuous in the nucleus. They progressively condense throughout zygotene and pachytene (Figures 20*B-D*), becoming localized among the chromosomes or in groups, often obscuring the chromosome relationships. These positively heteropycnotic bodies may still be observed associated with the chromosomes throughout pachytene and diplotene (Figure 20*C-E*) but gradually condense and often coalesce with the onset of diakinesis into a variable number of chromosome-like structures frequently with apparent chiasma formation (Figure 20*F*). This tentative ascription of the origin of the micro-chromosomes from heterochromatin associated with euchromatin in the prophase of meiosis is supported not only by similar evidence in somatic cells but by their ultimate fate in the final stages of sex cell maturation. In the second anaphase (Figure 21*K*) and especially in the final telophase, they are apparently resorbed into the macro-chromosome complex.

The micro-chromosomes often appear to coalesce and form aggregates approaching in size to the macro-chromosomes (Figure 20*E-G*) and sometimes they are barely visible (Figure 20*H-J*). That they possess a localized centromere is hardly credible, but that they divide is obvious. They constitute a real enigma to avian cytology and because of their anomalous number, morphology, behavior and segregation, as well as for other reasons to be divulged later in this paper, we have classed them as accessory or supernumerary chromosomes.

The nucleolus, which is very large in pachytene (Figure 20*C*), often persists, but with a considerable diminution in size, well into late diakinesis (Figure 20*J*). Its associated chromosome has not been identified. When observed at diakinesis, it has always been dissociated from its chromosome, perhaps as a result of the smearing pressure.

From first metaphase to sperm formation. At the reductional disjunction of metaphase I, the micro-chromosomes divide first (Figure 21*B-D*) and are often well into anaphase before the macro-chromosomes begin to separate. Whether the division of the former is qualitatively or quantitatively equivalent is questionable, for both by sectioning and the smear technique there usually occurs a partial confluence of the chromosomal sheaths of both the micro- and macro-chromosomes into sticky masses of varying numbers and sizes (Figure 21*B-E*). The precocity of the division of the smaller chromosomes or the relatively belated division of the larger chromosomes is shown in Figure 21*F*.

*Made by boiling 1 gram certified carmine in 100 cc of 45 percent propionic acid in a reflux condenser for 10-15 minutes. Cool, filter and use. Keeps indefinitely.



EARLY MEIOTIC STAGES IN THE FOWL

Figure 20

Figures 20, 21 and 22 are photomicrographs of smears taken at an initial magnification of 900× and enlarged in printing to 3000-3500×. *A*—Premeiotic interphase of primary spermatocyte showing chromocenter bodies which may be involved in micro-chromosome formation. *B*—Zygotene phase with heterochromatic bodies partially confluent with the chromosomes. *C*—Pachytene with nucleolus and nucleolus-forming chromosome attached. Chromocenter bodies ▶▶

The morphology of the six macro-chromosomes is only indifferently revealed at meiosis because of the greater degree of contraction of meiotic chromosomes. Their relative sizes are clearly shown in Figure 20*K*, but the position of the centromeres is better seen in Figure 21*A-E*.

The disjunction of the anaphase chromosomes at the reductional division is characterized by a precocious separation and poleward migration of the micro-chromosomes, followed by the shorter of the macro-chromosomes and usually last of all are the three largest chromosomes (Figure 21*F*), although this is not an invariable sequence. This sequence is apparently conditioned by the larger number of chiasmata of the larger chromosomes and the time required for their terminalization prior to anaphase separation. Figure 21*G* suggests an anaphase bridge just broken and a fragment lying alongside. Since such a configuration indicates a cross-over within an inverted chromosome, it may support the evidence from diakinesis for structural hybridity in this and perhaps other races of the domestic fowl.

Immediately upon the completion of primary anaphase (Figure 21*H*) the telophase chromosomes go directly into second metaphase without an intervening interphase or prophase (Figure 21*I*). This accords with Guyer's observations, but not with Miller's, who described resting and prophase stages prior to metaphase II. Anaphase follows swiftly (Figure 21*J* and *K*), judging from the relative infrequencies of their occurrence. The chromosomes at the second telophase (Figure 22*A* and *D*) begin to coalesce (Figure 22*B* and *C*), achieve a membrane (Figure 22*D* and *E*) and forthwith condense and elongate to form spermatozoa (Figure 22*F*).

Discussion

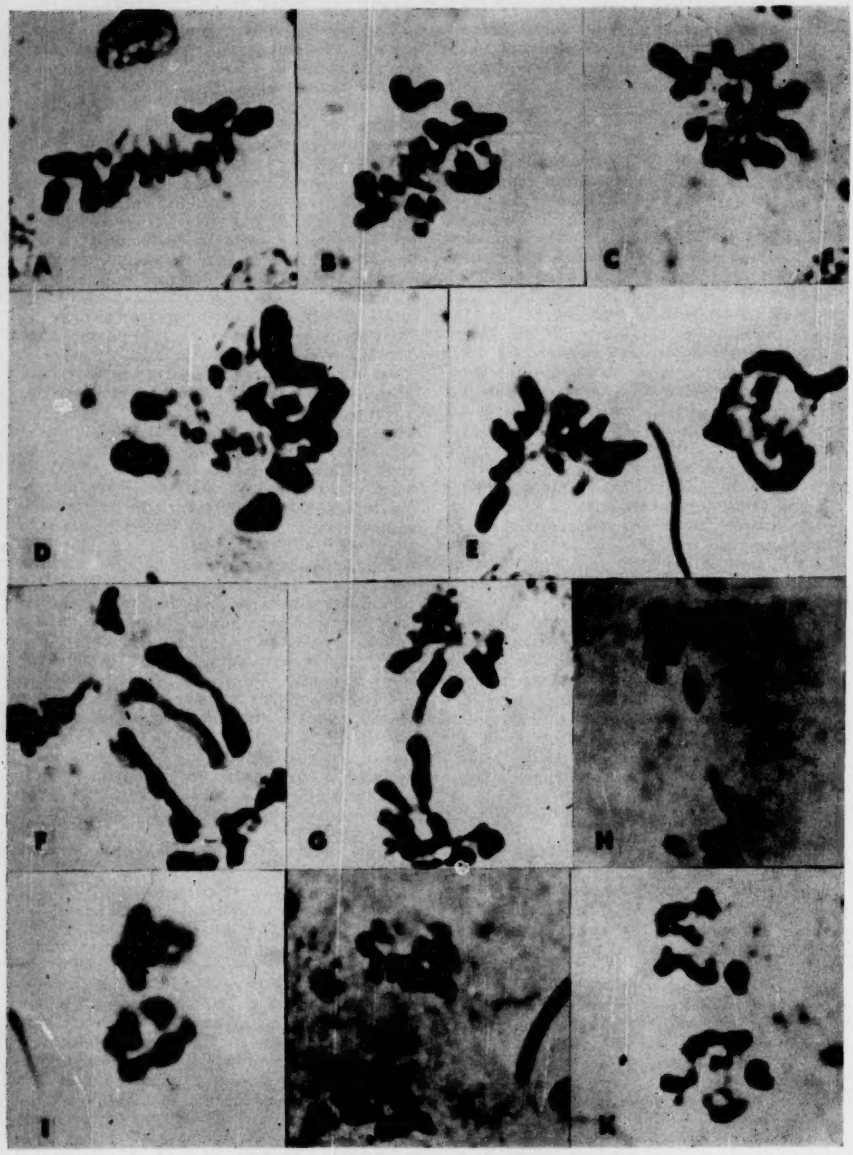
Chromosome number. Of the two previous investigators of spermatogenesis, Guyer[6] reported a chromosome number of "over 16 and not over 18" with eight or nine bivalents at meiotic metaphase. The micro-chromosomes were regarded by Guyer to be either linin, mitochondria, or fragmented chromosomes, probably the latter. Miller[19] reported that the chromosomes of the primary spermatocytes of the ovariotomized female are especially good for counting, and recorded them, showing 40 chromosomes, which would make a diploid number of 80, or 79 in the female since one of the elements is univalent according to Miller.

The results of our study on well spread smears of both primary and secondary spermatocytes in division not only do not support the trend to higher chromosome numbers in the fowl, but clearly indicate that the number of chromatin elements is variable throughout spermatogenesis (Figure 20*F*, *G*, *I*, *K*, 21*A* and 22*A*). This variability in number is apparently due to the anomalous behavior of the micro-chromosomes throughout meiosis. From observations of meiotic divisions it is seldom possible even to distinguish clearly between the macro- and micro-chromosomes, and our assumption made earlier in this report of six pairs of macro-chromosomes as constituting the large chromosome complement of the fowl is based partly upon somatic studies (unpublished—see also Figure 20*I* and *K*). More than six large bivalent elements are often present in the primary spermatocytes (Figure 20*F* and *G* and 21*A-D*) but since the lowest number of macro-chromosomes exhibiting normal behavior in both meiotic and mitotic divisions was six, we have adopted this minimal number for the macro-chromosomes. This figure also corresponds to the known number of linkage groups of the fowl.

The micro-chromosomes. From our experiences in duplicating the work of previous investigators using a sectioning technique and the recommended killing fluids, we do not believe it possible to achieve definitive results with either somatic or sexual tissue by these methods. The chromosomes of the fowl are not only small and numerous, but closely packed at the metaphase plate with the larger chromosomes on the periphery tightly enclosing the smaller units. For adequate examination, the chromosomes must be not only sharply stained but also spatially separated, which is possible only by a smear or squash technique.

Both the genetic and cytological evidence seem to require the designation of the micro-chromosomes of the fowl as accessory chromosomes. Genetic evidence supports it to the extent of demonstrating six linkage groups corresponding to the six pairs of macro-chromosomes. Exception to the genetic evidence consists of the presence of mutant genes not associated with any of the six linkage groups. The importance of this exception, especially in view of the reported variations of linkage values in some of the known linkage groups, cannot be evaluated at this time. In any event, the designation of the micro-chromosomes as accessories does not

condensing and associated with chromosomes. *D*—Partial figure of pachytene showing group of chromo-centers with some separating. *E*—Diplotene showing chiasmata. There are three chromosomes apparently primarily attached which are subtended by the nucleolus. *F*—Early diakinesis with the two chromosomes at the right in intimate association. *G*—Diakinesis with three chromosomes associated. *H-I*—Diakinesis with variable numbers of chromatic bodies. *J*—Shows clearly the six macro-chromosomes. The oval structure on the right is the nucleolus. *K*—Primary metaphase showing the six macro-chromosomes and the micro-chromosomes. The latter are variable in number (cf. *E-K*).



FIRST AND SECOND METAPHASE

Figure 21

A—Lateral view of first metaphase showing the large first and second curved chromosomes distinctly. *B*—Part of polar view in metaphase *I* shows precocity of division of smaller elements. The curved first chromosome at top resembles Miller's[19] description of the sex chromosome. *C*—Polar metaphase with the six macro-chromosomes enclosing the micro-chromo- ▶▶

necessarily imply that they are all genetically inert, although the cytological data would indicate a subsidiary role for them. Since the regular division of the chromosomes at mitosis and their regular segregation at meiosis are well established conditions of life and reproduction, and inasmuch as these conditions are met only by the six pairs of macro-chromosomes, it would seem to follow that the basic or fundamental genom of the domestic fowl is carried in the macro-chromosomes.

Further evidence from other organisms is provided in support of the accessory status of the micro-chromosomes. In appearance and behavior they are similar to the accessory chromosomes described in certain insects and worms,[18] and in the grasshopper and earwigs of White.[34] They also resemble the plant supernumeraries of corn,[4, 25] *Oenothera*,[3] *Clarkia*[14] and the mosses.[31] They exhibit a similar susceptibility to breakage (Figure 20*I-K*); they are heterochromatic though variable in staining (Figure 20*C, K* and 21*D*); they show an inclination to stickiness and fusion (Figure 21*B, C, E* and *F*); they are often precocious in division in both meiosis (Figure 21*A* and *F*) and mitosis (unpublished); they often fail to produce chiasmata in meiosis although some at times do (Figure 20*E-K*), and they are often allocyclic and heteropycnotic (Figure 20 *C* and *D*). In addition to the above, the behavior of the fragments and the fusion products during cell division suggest yet another distinction between the macro- and micro-chromosomes. The distinction is a structural one. The importance of the centromere or kinetochore in the chromosome cycle of division and anaphase migration has been known and emphasized in cytology since Navaschin's work in 1932 and the evidence for it need not be reviewed here. Suffice it to say that the usual fate of the acentric fragment of a broken chromosome or chromatid is to be lost in the cytoplasm unless restituted with its own or other unit possessing a kinetochore. It seems, therefore, that if the evidence for fragmentation of the micro-chromosomes of the fowl is valid, the possession of a kinetochore (at least a specific, localized one) is not necessary for their division and anaphase migration. That the evidence for the fragmentation of the micro-chromosomes is adequate is demonstrated by their range in size and number, and this is even better substantiated in somatic cells. It is also supported by the observations that only in the six pairs of macro-chromosomes can a primary constriction be detected, although the matter of size and visibility may enter here. Chromosomes with diffuse centromeres have been described in the coccid,[12] in the aphid[27] and in the plant *Luzula*,[15, 22] and their significance in the chromosomal evolution of the *Heteroptera* and *Homoptera* has been discussed by Schrader.[28] It appears, therefore, that the genom of the domestic fowl consists of two categories of chromosomes which differ in size, structure, behavior and, quite possibly, genetic significance. Such a disparate system could hardly maintain itself as a relatively true-breeding line if all its chromosomes were of equal genetic importance.

The sex chromosome. Our study revealed no clue to the morphological identity of the sex chromosome. Since genetic studies have shown that the female is heterozygous for sex and various investigators have reported the female to be XO in type, cytological observation might be expected to show a precocious meiotic anaphase movement of this chromosome in the female because it would be univalent in structure and have no chiasmata to resolve before leaving the metaphase plate. This is the type of evidence presented by Miller[19] to indicate that the metacentric fifth chromosome was identified with sex. From his published photographs, however, the evidence for this does not seem conclusive. The chromosome which he describes as being unpaired or univalent and slenderer in the female than its correspondent in the male, appears to us to be bivalent, and there is no evidence of precocity of anaphase migration (cf. his Figures 43, 44, 45). Miller's approach to the problem by inducing sex-reversal in a female and utilizing the spermatogenic gonad was excellent, but meiotic chromosomes, because of their greater contraction than mitotics, are poor material for morphological study. It seems clear that the solution to this problem must await better technical studies on the somatic chromosomes of both sexes.

Genetic data might seem to support the earlier work of Guyer[5, 6] and Hance[7, 8, 9, 10, 11] for the first or largest chromosome being associated with sex, although this element is not unpaired in the female. Warren[32] observed that some of the sex-linked factors in the fowl show what appears to be independent assortment among themselves. This suggested to Warren that map distances on the sex chromosome are so great that the percentage of crossing-over is large enough to obscure the linkage evidence. This, however,

somes which are partly fused with each other. *D*—Polar metaphase with seven large elements—the seventh representing a fusion product of micro-chromosomes. *E*—In the left figure, six macro-chromosomes are visible; in the right figure all elements are confluent. *F*—First anaphase with the three largest chromosomes still separating while the smaller elements have reached the poles. *G*—Late anaphase suggesting a bridge just broken with a fragment alongside upper member. *H*—First telophase with one chromosome slightly lagging. *I*—Second metaphase arising directly from first telophase. *J* and *K*—Second telophase.

TELOPHASE TO SPERMATOGENESIS

Figure 22

A—Second telophase. *B*—Spermatids from second telophase coalescing and elongating to form sperm. *C*—A continuation of *B*. A tail has appeared on one spermatid. *D*—Four spermatids showing developmental sequences toward sperm formation. *E* and *F*—Spermatids developing into spermatozoa.

is difficult to reconcile with the fifth chromosome. This chromosome, by reason of its small size and short arms, is perhaps the least favorable of the complex for the occurrence of multiple cross-overs. The simplest assumption seems to be that the sex chromosome or the expression of sex is associated with one of the longer J-shaped structures where chiasmata frequency is high, and that linkage relations are obscured by multiple cross-overs, heterochromatin, structural hybridity or other causes.

Summary and Conclusions

A propionic-carmine smear technique in conjunction with a new killing fluid has been used in this study of avian chromosomes.

As a result of our observations we have been led to separate the chromosomes of the domestic fowl into two categories: the macrochromosomes and the accessory chromosomes. These categories differ in morphology, size, behavior, structure and also, perhaps, in genetic significance.

The chromosome number of the fowl is not constant because of the fusion and fragmentation exhibited by the accessory chromosomes. The accessory chromosomes are largely heterochromatic and may not possess a discrete kinetochore.

This study is to be followed by one on the

somatic chromosomes of the same breed of fowl.

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GAIN IN WEIGHT OF PREGNANT MICE

In Relation to Litter Size

ELIZABETH FEKETE*

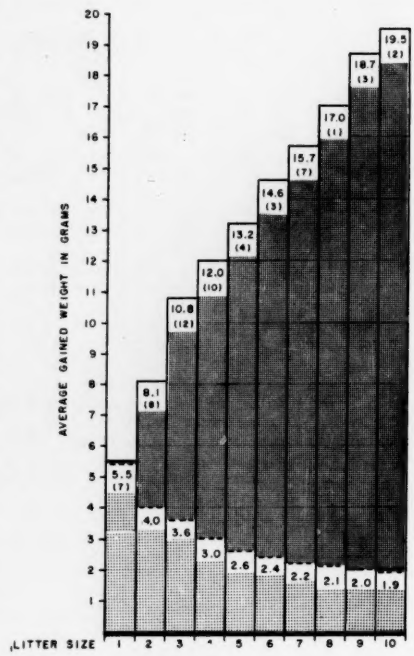
DATA on the influence of litter size on birth weight of mice are available in the literature. The most accurate of such data are those reported by Crozier and Enzmann[1] who, using Bagg albino mice, recorded the weight of first litters by weighing the unfed newborn young. They showed that a single newborn weighs 1.63 grams while the average weight of a new-born in a litter of 10 young is 1.14 grams.

Data are evidently lacking in the literature on the gain in weight of the mothers during pregnancy as influenced by litter size.

This paper presents data on the gain in weight of 57 pregnant C57BL/6 females gravid with litters varying in size from one to 10. The data were not collected for the primary purpose of determining gain in weight during pregnancy but were a by-product of experiments involving the transfer of fertilized ova from other inbred strains into the uteri of C57BL mice. However it can be assumed that the experimental procedure had no effect on the gain in weight for the following reasons: the animals of strains used as donors of ova do not differ in size from those of the C57BL strain; in about half of the animals the transfer of ova was unsuccessful and only C57BL young were born. No difference was noted between those animals that gave birth to some young from the transferred ova and those that gave birth to C57BL young only.

Before being used for timed matings, weaning age females were kept with males until they became pregnant and were separated from the males prior to giving birth to their first litters. Since the onset of estrus and mating both occur most frequently between 10 p.m. and 1 a.m. (Snell *et al*[3]), for timed matings a fertile male was placed in a cage with several females in the late afternoon and the females were examined for vaginal plugs the next morning.

Weighing was started the third morning after observation of the plug and the weight of the mouse on this day was considered the initial weight. Thereafter the animals were weighed daily at approximately the same time. The morning of the 19th day after observation of the plug was the last weighing and the difference between the initial weight and this weight was considered the total gain.



AVERAGE GAIN

Figure 23

Data on the gain in weight during pregnancy of C57BL/6 mice. The columns represent the average gains in grams. The numbers in parenthesis show how many gravid females were weighed in each group. The lightly shaded areas show how much gain was contributed by one young in each litter-size group.

It was expected that the young would be born on the 20th day. According to Merton[2] the maximum number of births occur between midnight and 4 a.m. If the litter was born on the expected day the number of young was recorded around 8 a.m. If the litter was not

(*Continued on page* 98)

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This work is part of a project aided by the American Cancer Society upon recommendation of the Committee of Growth of the National Research Council and in part by the National Cancer Institute of the National Institute of Health, Public Health Service.

EFFECTS OF INBREEDING

On a Flock of Ossimi Sheep

M. T. Ragab and A. A. Asker*

INBREEDING reports on livestock come mainly from studying herd books or closed flocks to outside blood. Recently, reports have been published on actual inbreeding experiments on cattle and pigs in the United States. Such experiments have yielded information on the effect of inbreeding on weight, fertility and other economic characteristics. During the course of some of these experiments lethals and undesired traits have been brought to light.

The present paper reports the effect of inbreeding on birth weight, weaning weight and marketing weight in a closed flock of Ossimi sheep. Some animals were born blind in this flock, and it was interesting to investigate the genetic nature of this defect. Blindness was not among the defects reported by Eaton[3] and Lerner[7] in sheep.

Material and Methods

Weight records covering a period of 10 years (1940-1950) of the Ossimi flock of sheep kept by the Animal Breeding Department, Faculty of Agriculture, Giza, were used in this study. The flock was originated in 1934 and kept closed to outside blood since 1939. Lambs were weighed 12 hours after birth and every two weeks until they reached weaning age (four months). After weaning, animals were weighed every month and usually marketed at six months of age. Kady,[6] studying causes of variation in weights of sheep in this flock, showed that sex of the lamb, type of birth and sire of the lamb were the main factors affecting weights significantly. Therefore, our analyses have been worked out for each sex and type of birth separately and on an intra-sire basis to eliminate the effect of sire. The formula developed by Wright[15] was used in estimating the coefficient of inbreeding.

Results and Discussion

1. Effect of Inbreeding on Weights

The intra-sire regressions of birth weight, weaning weight and marketing weight on inbreeding as well as the correlation coefficients between the different weights and inbreeding are shown in Table I. The analysis showed that inbreeding was responsible for decreasing the three weights of single lambs of both sexes. On the other hand, the regression coefficients obtained for twin males and females at different ages were not statistically significant.

The data indicate that the decrease in weights due to inbreeding was more pronounced at marketing age than at either birth or weaning time. Effect of inbreeding was also more noticeable in the case of males than females.

The weighted average of the male and female regressions showed a decrease of about one-tenth and one-fifth of a kilogram in weaning and marketing weights respectively for each increase of one degree of inbreeding. In the case of single lambs, the average decline at birth was about 26 gm. for every one percent increase in inbreeding.

On the basis of these results, one should expect a decrease of about 3.1 and 4.8 kgs. at weaning and marketing weights of single lambs respectively for one generation of parent-offspring mating. A first generation of full-sibs mating would be expected to lower the average birth weight of single lambs by 16 percent.

The results are in concordance with those reported by other investigators. Hazel and Terrill,[5] Glembocki and Nahimison[4] and Terrill *et al*,[10, 11] have found that inbreeding was responsible for decreasing birth weight and weaning weight of sheep. White[13] stated that inbreeding has resulted in a reduction of 0.278 lb. of body weight for each 1 percent increase in the degree of inbreeding. Also reports on pigs by Comstock and Winters,[2] Whately[12] and Winters *et al.*[14] pointed out that inbreeding had an adverse effect on weights at different ages.

The effect of inbreeding of the dam on the birth, weaning and marketing weights of single lambs was also studied. Our analysis indicated that the dam's coefficient of inbreeding did not affect the weight of her offspring significantly.

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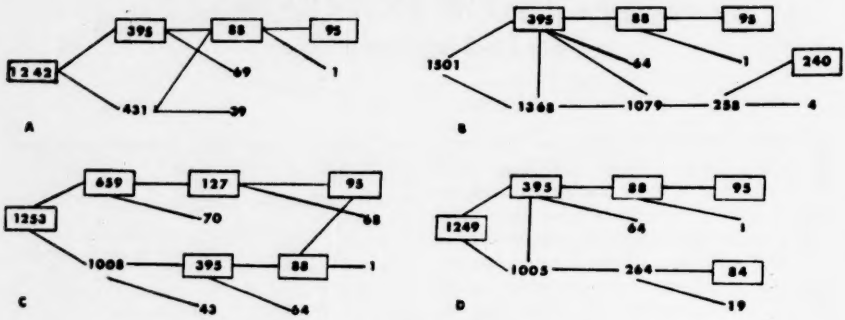

PEDIGREES OF INBRED OSSIMI SHEEP

Figure 24

Coefficients of inbreeding range from 12.5 for *A*, 37.5 for *B*, 1.56 for *C* and 25.0 for *D*.

It is clear from this investigation that mild inbreeding caused a significant decline in the birth, weaning and marketing weights of single lambs. Probably this reduction in weight may affect vigor, fertility and other economic characteristics of animals. Although stock rams used in the flock were selected on the basis of their superiority in weight and growth rate, such procedure did not offset the adverse effect of inbreeding.

2. *Occurrence of Blindness*

Although the flock was founded in 1934, no cases of blindness or any other defects were noticed until 1945. In fact, in 1939 a ram purchased from outside (No. 95) was used extensively on the flock. Its first crop of lambs was dropped in 1940 and since that time all the stock rams used were its own progeny. Ram No. 395, a grandson of ram No. 95, was the most widely used of all rams in the history of the flock and stayed for seven years.[9]

There were 15 cases of blind animals born by 1950, seven females and eight males. All the defective animals were inbred and the average coefficient of inbreeding for these animals was 25.5 percent. Most of them were produced through sire-daughter matings and the common ancestor in the pedigree of all defective animals was ram No. 95 (Figure 24).

The defect (blindness) is easily recognized in new-born lambs, since the defective animals do not easily take to their dams, and usually lose them if not guided. The death of such animals, unless they receive great care all through their life, is inevitable. In fact, their life is shorter than that of their normal mates; they died because of anemia and general debility.

In analyzing the pedigrees of the defective animals, blindness seems to be due to a simple

TABLE I. Effect of Inbreeding on birth weight, weaning weight and marketing weight.

Type and sex of animals	No. Animals	No. Sires	Av. Weight	Av. Inbreeding	Intra - Sire	
					Regression	Correlation
Birth Weight.			Kg.	%		
Single Males	189	9	3.99	5.68	—0.0288†	—0.3794†
Single Females	170	8	3.79	6.08	—0.0239†	—0.2388†
Twin Males	88	9	3.32	7.89	—0.0123	—0.1936
Twin Females	87	6	3.01	8.07	—0.0049	—0.1273
Weaning Weight.						
Single Males	189	9	23.81	5.11	—0.1653†	—0.3112†
Single Females	170	8	22.26	5.66	—0.0968*	—0.1494*
Twin Males	80	8	20.08	7.54	—0.0538	—0.1339
Twin Females	87	7	17.29	7.64	—0.0324	—0.0921
Marketing Weight.						
Single Males	168	9	31.83	4.56	—0.2440†	—0.4188†
Single Females	131	6	29.42	4.80	—0.1155*	—0.1976*
Twin Males	59	8	27.77	6.95	—0.1354	—0.2301
Twin Females	68	7	24.28	6.05	—0.0496	—0.1273

* $P < 0.05$ † $P < 0.01$

recessive autosomal pair of genes (*bb*). Ram No. 95 was responsible for introducing this undesirable gene.

As the number of defective animals was 15, and the total number of offspring produced by ram 95 and its progeny came to 854 lambs, the percentage of blind individuals was 1.75 percent. Since defective animals were not used for breeding and rams used on the flock were heterozygous for the defect (*Bb*), the mating practiced in this flock would therefore be of the following types:

	Offspring	
Type of Mating	Normal	Blind
$BB \times Bb$	$4P^3q$	——
$Bb \times Bb$	$3P^2q^2$	P^2q^2

Consequently, the number of blind individuals in the whole population will be equal to

$$\frac{P^2q^2}{4P^3q + 4P^2q^2} = \frac{q}{4}$$

Since $\frac{q}{4} = 0.0175$, then the frequency of (*b*) will be 0.07 in this population. It was not possible to know the proportion of the genotypes *BB* and *Bb* in the population, since testing with the double recessive (*bb*) was not possible.

According to these results, one could predict how selection against this undesirable gene, under random mating would change its frequency. Since the genetic interval for the Ossimi sheep is 4.00 years,[1] it is expected that the frequency of gene (*b*) will be changed from 0.07 to 0.061 after 80 years of elimination of defective animals.[8] However, this change is very small and slow to be of any practical value. To purify the flock from this defect in a short time, each individual should be tested to differentiate the normals (*BB*) from the carriers (*Bb*). This of course would require the maintenance and use of blind animals for testing.

Summary

Weight records of a closed Ossimi flock of sheep belonging to the Animal Breeding Department, Faculty of Agriculture, Giza, covering a period of 10 years (1940-1950) were analyzed. This study was carried out to investigate the effect of inbreeding on birth weight, weaning weight and marketing weight of lambs.

It has been found that inbreeding was responsible for a significant reduction in the weights of single lambs. On the other hand, the effect of inbreeding on the weights of twin lambs was not statistically significant. Inbreeding of the dam had no significant effect on the weight of her offspring.

Fifteen blind lambs were born during the last five years of the period of study. This defect was due to a pair of autosomal recessive genes. The frequency of this gene was estimated as 0.07.

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COMPUTATION OF INBREEDING AND RELATIONSHIP COEFFICIENTS

In Populations with a Relatively Small Number of Different Male Ancestors

MOGENS PLUM*

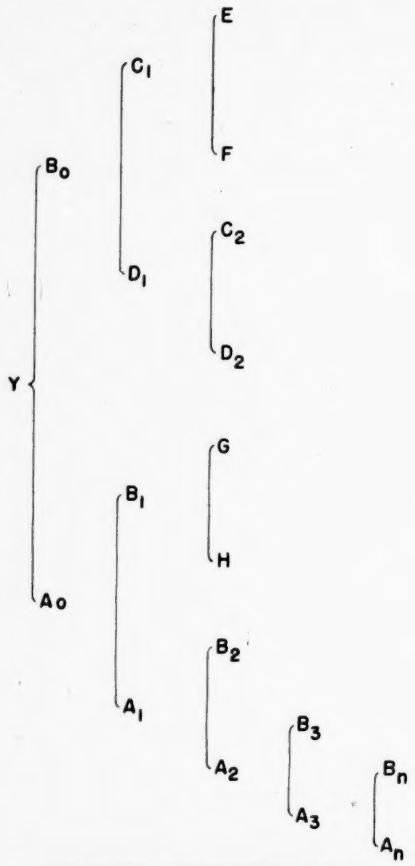
THE computation of inbreeding and relationship coefficients as formulated by Wright[4] involves the tracing of all the paths which connect two individuals through a common ancestor. Wright's formula for the inbreeding of an individual x is

$$F_X = \Sigma [(\tfrac{1}{2})^{n+1} (1 + F_A)]$$

in which n is the number of individuals in a path connecting egg and sperm and passing through ancestry A of these two individuals and F_A is the inbreeding coefficient of the common ancestor. The formula shows that the coefficient of inbreeding of an individual is simply ½ of the genic covariance between the individual's sire and dam.[3]

Various simplified methods have been described to compute the inbreeding and relationship coefficients.[1,2,3] The method outlined by Hazel and Lush[3] by which punched cards may be used to compute accurate values of the genic covariances between two individuals has removed from these computations some of the mental gymnastics necessary to carry out correct path-tracing operations. The method of Cruden[1] may be employed to compute covariance charts involving all the animals in a closed population and makes it unnecessary to trace any paths once the covariances among the foundation animals have been computed.

In spite of these valuable aides in computation, many populations soon reach such a size that the actual handling and computation of covariance charts become major operations. In many cases inbreeding and relationship coefficients may, however, be computed quite rapidly from covariance charts involving only: 1) the mates to the females in the direct female line of ancestry, often referred to as the bottom of the pedigree; and 2) the females that have female descendants represented in the population and at the same time are



PEDIGREE OF INDIVIDUAL Y

Figure 25

The pedigree of individual Y represented in the conventional form.

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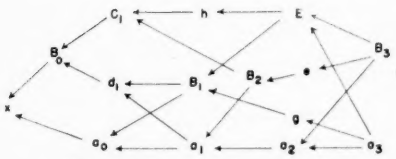


PEDIGREE OF INDIVIDUAL X

Figure 26

Arrow pedigree of individual x. Males are indicated by capital letters, females by small letters.

ancestors of one of the mates. The number of these females is often very small. The present paper describes this simplified method and illustrative examples are given.

Outline of Method

Figure 25 represents a pedigree of conventional form. The inbreeding of the individual Y is (cov B_0A_0)/2, but according to Cruden's paper[1] cov B_0A_0 = (cov B_0B_1)/2 + (cov B_0A_1)/2. By extension of this principle we find that cov B_0A_1 = (cov B_0B_2)/2 + (cov B_0A_2)/2, and proceeding with this expansion, we arrive at the general formula which is

cov B_0A_0 = (cov B_0B_1)/2 + (cov B_0B_2)/4 + ... (cov B_0B_n)/2^n + (cov B_0A_n)/2^n (1)

where B_1— B_1— B_2 . . . . —B_n are the mates to the females in the direct female line at the bottom of the pedigree (A_0— A_1 . . . . A_n).

Formula (1) indicates that only the direct female line together with their mates needs to be traced in order to compute the inbreeding coefficient of individual Y. Since all calculations of inbreeding coefficients are relative to some base date, the direct female line needs only to be traced back to this base date after which the term (cov B_0A_n)/2^n may be dropped from the formula in most cases.

The method has one limitation. Whenever one of the A-animals is also an ancestor of one of the B-animals, the computation cannot be carried back of the particular A-animal in question. For instance if A_3 is a common ancestor to both A_2 and B_0 the computation cannot be carried back to A_2. The genic covariance between B_0 and A_3 must be computed and the complete formula for the genic covariance between A_0 and B_0 will be:

cov B_0A_0 = (cov B_0B_1)/2 + (cov B_0B_2)/4 + (cov B_0B_3)/8 + (cov B_0A_3)/8 (2)

In most cases the last term of this formula may be computed by going back of the individual which is not a common ancestor (B_0) because cov B_0A_3 = (cov A_3C_1)/2 + (cov A_3D_1)/2 and this formula may be further expanded according to the principle of formula (1).

Procedure for Computation of Inbreeding Coefficients

When using this procedure, the first step is to tabulate the direct female ancestry together with their mates for each animal whose inbreeding coefficient is to be computed. Once this is done the actual covariance chart will be limited to the males appearing in these female ("family") pedigrees. If individuals which are (female) ancestors of some of the males appear in any of the "families," these "foundation" females should also be included in the covariance chart. When a covariance chart has been computed, the inbreeding of any individual or the relationship between any two individuals may be computed by means of formula (1) or (2).

The covariances between the males and between the males and the foundation females may be computed by the use of punched cards according to the method outlined by Hazel and Lush.[3] If however, the males are bred within the herd or population under study, it may be simpler to start with the foundation males and females and work forward according to the principle outlined by Cruden[1] using either formula (1) or (2) of this paper.

Example

Figure 26 gives the arrow pedigree of an individual x. The pedigree is drawn as an arrow pedigree mainly to save space. All the males are indicated by capital letters and the females by small letters. In the case of animal B_0 the "bottom" of the pedigree would be: d_1 . . a_1 . . a_2 . . a_3 with the mates C_1 . . B_1 . . B_2 . . B_3 In order to limit the number of illustrations, Figure 26 has been drawn so that all the males and the foundation females are included in this one pedigree This would rarely happen in actual data but by means of this illustration it is possible to demonstrate the computation of the covariances given in Table I. Starting from the top left corner of the table the covariances are all very easy to compute from formula (1) or (2). For instance cov B_0B_1 = (cov B_1C_1)/2 + (cov B_1B_1)/4 + (cov B_1B_2)/8 + (cov B_1B_3)/16 + (cov B_1a_3)/16.

The numerator values may all be read off from the previous computations recorded on the chart, and cumulative multiplication of the

TABLE I. Genic covariances between individuals in arrow pedigree. (Figure 26)

	a_3	B_3	E	B_2	B_1	C_1	B_0	x
a_3	1.0000							
B_3	0000	1.0000						
E	5000	5000	1.0000					
B_2	0000	2500	1250	1.0000				
B_1	5000	2500	6250	0625	1.1250			
C_1	1250	2500	3125	5312	1875	1.0312		
B_0	2500	2812	3906	4219	4297	6523	1.1367	
x	3125	2969	4297	3672	5508	4629	8008	1.2324

values for $B_1 C_1 \ldots B_1 B_1 \ldots \ldots B_1 a_3$ by the fraction 0.50 . . 0.25 . . . . 0.0625 gives the final answer $0.1875(0.50) + 1.125(0.25) + 0.0625(0.125) + 0.25(0.0625) + 0.50(0.0625)$ which is 0.4297.

When a covariance chart including all the males represented in the pedigrees under study has been completed, the computation of the individual inbreeding coefficients is simple. Figure 27 shows two sample pedigrees of individuals "i" and "n" whose inbreeding coefficients are to be computed. The pedigrees are traced back through the direct female line (small letters) and include only these females and their respective mates (capital letters). The coefficient of inbreeding for individual "i" is $(\text{cov } B_0 j)/2$, but $\text{cov } B_0 j = (\text{cov } B_0 C_1)/2 + (\text{cov } B_0 B_2)/4 + (\text{cov } B_0 B_3)/8$ which according to Table I equals $0.6523/2 + 0.4219/4 + 0.2812/8 = 0.4668$ giving a coefficient of inbreeding for individual "i" of 0.2334.

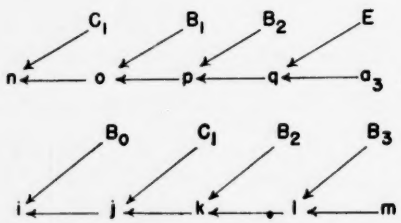
The inbreeding coefficient of individual "n" is $\text{cov } C_1 0/2$. However, individual "n" is a direct female descendant of a_3 for which reason we have $\text{cov } C_1 0 = (\text{cov } C_1 B_1)/2 + (\text{cov } C_1 B_2)/4 + (\text{cov } C_1 E)/8 + (\text{cov } C_1 a_3)/8$. This according to Table I is $0.1875/2 + 0.5312/4 + 0.3125/8 + 0.1250/8 = 0.2812$, giving a coefficient of inbreeding for individual "n" of 0.1406.

Sometimes slightly more complicated computations are necessary. The computation of the covariance between x and B_0 in the pedigree illustrated in Figure 26 will be used as an example. Individual a_1 is a common ancestor to both x and B_0, but is not included in the covariance chart (Table I). Table II illustrates one method by which the computation may be carried out from the values given in the covariance chart given in Table I. The covariance between x and B_0 is equal to $(\text{cov } B_0 B_0)/2 + (\text{cov } B_0 B_1)/4 + (\text{cov } B_0 a_1)/4$. This is entered in tabular form in Table II, line 1 and 2. Because a_1 is an ancestor common to x and B_0 it is not possible to extend the formula back of a_1. However, $\text{cov } B_0 a_1 = (\text{cov } a_1 C_1)/2 + (\text{cov } a_1 B_1)/4 + (\text{cov } a_1 a_1)/4$ and consequently the three values on the right hand side of this equation are entered in Table II in place of $\text{cov } B_0 a_1$. This is indicated in lines 2, 4 and 5. Covariances involving a_1 must, however, be eliminated and therefore $(\text{cov } C_1 B_2)/2 + (\text{cov } C_1 B_3)/4 + (\text{cov } C_1 a_3)/4$ is substituted for $\text{cov } a_1 C_1$ as indicated in lines 2 and 3, and $(\text{cov } B_1 B_2)/2 + (\text{cov } B_1 B_3)/4 + (\text{cov } B_1 a_3)/4$ is substituted for $\text{cov } a_1 B_1$ as indicated in lines 4 and 5. The values from Table I may now be entered in their appropriate places in Table II. The only value not found in Table I is $\text{cov } a_1 a_1$. For a population mating at random the covariance of an individual with itself is 1. The general case of the covariance of an individual (a) with itself is $(1 + F_a)$. The inbreeding of a_1 is $(\text{cov } B_2 a_2)/2$ which equals $(\text{cov } B_2 B_3)/4$. The value for $\text{cov } B_2 B_3$ may be found in Table I, giving a value for $\text{cov } a_1 a_1$ of 1.0625. After the values are all entered in Table II the final computation is $1.1367/2 + 0.4297/4 + 1.5938/16 + 0.4375/32 + 0.7500/64$ which equals 0.8008. This is the genic covariance between x and B_0. If the relationship coefficient between x and B_0 is to be computed, the genic covariance must be corrected for the inbreeding of x and B_0 according to Wright's formula.[4]. The relationship coefficient becomes $0.8008/\sqrt{1.1367 \times 1.2324} = 0.6766$.

TABLE II. Computation of covariance × B_0 from values given in Table I

Line	FACTOR					
	50	25	125	0625	03125	015625
1	$B_0 B_0$ 1.1367	$B_0 B_1$ 0.4297				
2		$B_0 a_1$	$a_1 C_1$	$C_1 B_2$ 0.5312	$C_1 B_3$ 0.25	
3					$C_1 a_3$ 0.125	
4				$a_1 B_1$	$B_1 B_2$ 0.0625	$B_1 B_3$ 0.25
5				$a_1 a_1$ 1.0625		$B_1 a_3$ 0.50
TOTAL	1.1367	0.4297		1.5937	0.4375	0.75

Sum (column x factor) = 0.8008



FEMALE LINE PEDIGREES

Figure 27

"Female line" pedigrees of individuals "i" and "n" whose coefficient of inbreeding are to be computed.

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THE GENETIC HISTORY OF THE ARABIAN HORSE

In the United States

P. J. GAZDER*

THE Arabian horse is among the oldest of the breeds bred by man for his use, and the influence of the Arabian is also felt in many other light horse breeds, among them the Thoroughbred, the American Saddle horse, the Quarter horse, and the Palomino. The breed originated in the desert of Arabia among the warring tribes, and gradually spread, first to neighboring countries, and later to far distant ones in both hemispheres. It is a small breed and has been unique among horse breeds in that it is "the only breed in which no outcross is permitted, or has been permitted in historic times," (J. M. Dickinson, Traveller's Rest Stud Catalog. 1947). Arabian horse production is consequently limited to the purebred progeny of Arabian mares, and only these are eligible for registry in the Arabian Horse Club Registry of America, the registering body in the United States. All living registered Arabian horses descend from animals imported since 1879. Purebred Arabians were imported before this date but were used in the development of the American Saddle Horse, the Standardbred, and the American Thoroughbred, and their influence was lost to the pure breed. Forty-five Arabian stallions and 21 Arabian mares were imported between 1760 and 1906, the year in which the Stud Book of the Arabian Horse Club was established.

Most of the imported animals came from herds breeding the same or closely related bloodlines, and so were related to one another. This was due both to the small size of the breed, and to the breeding practices followed by breeders to increase the influence of superior animals by adopting programs involving inbreeding or linebreeding. Unlike most horse breeds, the Arabian has not been selectively bred for some specific ability, such as speed or show-ring performance, but has been selected for soundness and general over-all ability including action, endurance, docility and beauty.

The early genetic history of the Arabian horse, was studied by Zaher[5] in an analysis of registered animals during the period from 1906, when the Arabian Horse Club Registry was established, to 1946. He found that inbreeding increased from 1 per cent in 1907-1916 to 4.8 percent in 1937-1946. The *inter se* relationship was 1 percent in 1907-1916, 4 percent in 1917-1926, 2 percent in 1927-1936, and 4 percent in 1937-1946. Coefficients of inbreeding were higher than the expected from *inter se* relationship in a finite group mated at random, indicating the tendency toward the formation of separate families.

Generation Interval Study

The animals were divided into seven periods, which contained approximately 600 animals in each period, with the exception of period three which contained approximately 2600 animals. Intervals between foal and dam, foal and sire, and the average were determined from samples of 20 drawn randomly from each period. The results are shown in Table I.

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The interval between sire and offspring was almost always greater than the interval between dam and offspring, and this was at least in part due to the prevalent practice of Arabian breeders of using their older and proven sires as long as they remain capable of service. Animals of this breed are capable of remaining in productive life until very advanced ages; e.g., Nejdme 1, the first animal registered in the Stud Book bore her last foal at the age of 32.

Genetic Studies

Inbreeding and Inter-se Relationship

The methods of breed analysis developed by Wright[4] and adapted to punched cards by Hazel and Lush,[2] were used in this study.

A random sample of 10 animals was drawn from each of the four years, 1947, 1948, 1949, 1950. A show sample was also constructed by considering the first place winners in the breeding classes of "yearling," "2 years," "3 years," "4 years and over" at the National Stallion show in Iowa, and the All Arabian Show at Pomona, California. A total of 36 animals, from the years 1949, 1950 and 1951 constituted the show sample.

The inbreeding and *inter se* relationship coefficients for the years 1947, 1948, 1949, 1950 and two samples of show winners are shown in Table II.

The period of 1947-1950 was a period of rapid increase for the breed during which it nearly doubled its numerical strength. Warden[3] observed that the Arabian had made the greatest gain of any breed in yearly registrations of new animals between 1942 and 1950. The values for inbreeding and *inter se* relationship were lower than those found for the period immediately preceding, (1937-1946), by Zaher,[5] Table III. This may be accounted for by the extensive use of all young stock and the formation of new establishments using purchased animals of divergent bloodlines, as well as the introduction of "new blood" from Europe and the Middle East.

The show system of the Arabian horse was first criticized by Du Flon[1], who noted that there were "no Arabian horse show circuits," but that there were two main groups of exhibitors, one on the Pacific Coast, the other in Ohio, Illinois, and Tennessee. Zaher has also observed the deficiency of sufficient All Arabian Shows to study the breeding systems used in producing outstanding animals. This need still exists, although there is a growing awareness of the deficiency of suitable horse shows of sufficient representation. An attempt was made to create a representative show sample for this study by utilizing first place winners from two popular shows, one Midwestern and one Far Western in geographical location. Animals of the National Show were more highly inbred than the average of the breed, while animals of the Pomona Show showed considerably less inbreeding but had a high *inter se* relationship.

Influence of Specific Animals

The relationship of specific animals to the breed during the period 1947-1950 is shown in Table IV. The animal with the highest relationship to the breed was the stallion Skowronek who had a relationship of 2.06 percent to the breed. He influenced the breed chiefly through his sons Raffles 952 and Raseyn 597, his daughter Rifala 815, and their progeny. Hamrah 28, who had the highest relationship to the breed in Zaher's study, dropped to second place. He had a relationship of 2.0 percent to the breed, chiefly through his daughters Moliah 109 and Sankirah 149 and his grandsons Hanad 489 and Antez 448. Nasik 604 held third place with 1.97 percent while Mesaoud, through his many descendants,

TABLE I. The interval (years) between generations in Arabian Horses

Type of Interval	Period							
	1906-1926	1927-1936	1937-1946	1947	1948	1949	1950	1947-1950
Sire to offspring	12.15	13.20	10.22	12.70	9.65	7.65	10.65	10.16
Dam to offspring	10.70	10.70	9.78	9.15	9.90	9.45	8.20	9.18
Average	11.43	11.95	10.00	10.93	9.78	8.55	9.43	9.67

TABLE II. Inbreeding and *inter se* relationship coefficients (%)

	1947	1948	1949	1950	Average of Years	Show (N)*	Show (P)†	Average of Shows
Average Inbreeding (%)	1.70	2.84	2.62	1.47	2.16	3.26	0.10	1.68
inter-se Relationship	2.70	5.62	4.25	0.38	3.26	2.01	3.62	2.82

* N = National Stallion Show, Iowa. (1949, 1950, 1951)
† P = All Arabian Show, Pomona, California. (1949, 1950, 1951)

took fourth place with 1.72 percent.

The female with the highest relationship to the breed was Ferda 596, a foundation mare of the W. K. Kellogg Institute, who had a relationship of 1.44 percent to the breed. Narghileh, through her sons Nasik 604 and Nureddin II 974, had a relationship of 1.19 percent, while Rifala 815 had a relationship of 1.06 percent chiefly through her sons Raffles 952 and Rifage 1286.

The relationship of specific animals to show winners is shown in Table V. Among the stallions, the first five places are occupied by members of two lines, the Skowronek and Mirage lines. The Skowronek line is a strong influence, for Skowronek, his sons Raseyn, Raffles, and Naseem, his grandsons Ferseyn, Image, and Phantom, and his great-grandsons Gaysar and Abu Farwa are all found in the table. In addition, his daughter Rifala heads the list of important mares. The Mirage line is also prominent in the production of show winners, and crosses well with the Skowronek line. Egyptian bloodlines have produced many good dams of show winners such as Bint Bint Sabbah, Ralouma, Fadahma, and Rageyma.

TABLE III. Inbreeding and *inter se* relationship coefficients (%)

No.	Period*	*inter-se* Relationship %	Inbreeding Actual	Coefficients Expected†
1	1907-1916	1.0	1.0	0.5
2	1917-1926	4.0	2.0	2.0
3	1927-1936	2.0	1.8	1.0
4	1937-1946	4.0	4.8	2.0
5	1947-1950	3.3	2.2	1.7

* Periods 1 to 4 are from Zaher.[5]

† Expected inbreeding $= \frac{inter\ se\ \mathrm{R}}{2 - inter\ se\ \mathrm{R}}$

TABLE V. Relationship (%) of specific animals to show winners

STALLIONS	SKOWRONEK	3.97
	RASEYN 597	2.69
	MIRAGE 790	2.00
	FERSEYN 1381	2.00
	IMAGE 1008	2.00
	HAMRAH 28	1.66
	ABU FARWA 1960	1.50
	ALLA AMARWARD 1140	1.50
	RUSTEM	1.42
	REHAL 504	1.34
	RABIYAS 1236	1.25
	MESAOUD	1.21
	FADL 896	1.12
	RAFFLES 952	1.12
	NASEEM	1.12
	ANKAR 3063	1.00
	ANTEZ 448	1.00
	GAYSAR 2387	1.00
	GULASTRA 521	1.00
	PHANTOM 2186	1.00
MARES	RIFALA 815	2.38
	REHASAFA 1269	1.50
	FERDA 596	1.34
	RAYYA	1.34
	RISSLETTA 1201	1.25
	RISSLA	1.18
	FARHAN 684	1.12
	BINT BINT SABBAH 894	1.00
	BINT SAHARA 2394	1.00
	FADAHMA 1245	1.00
	RAGEYMA 1289	1.00
	RALOUMA 1132	1.00

Summary

A study was made of the Arabian Horse in the United States and the resultant information may be summarized as follows:

1. The average generation interval was 11.43 years in 1906-1926, 11.95 years in 1927-1936, 10.0 years in 1937-1946, and 9.67 years in 1947-1950.

2. The average inbreeding for the years 1947, 1948, 1949, 1950 was 1.70 percent, 2.84 percent, 2.62 percent, and 1.47 percent, respectively. The average *inter se* relationship was 2.70 percent, 5.61 percent, 4.25 percent and 0.38 percent, respectively. Expected inbreeding (computed from the *inter se* relationship and assuming random mating) was in most cases considerably smaller than the actual value, indicating that an appreciable amount of deliberate inbreeding has been practiced.

3. Show winners had high *inter se* relationship, and winners at the National Stallion Show were more highly inbred than winners at the All Arabian Show, Pomona, California.

TABLE IV. Relationship (%) of specific animals to the breed

STALLIONS	SKOWRONEK	2.06
	HAMRAH 28	2.00
	NASIK 604	1.97
	MESAOUD	1.72
	CZUBUTHAN 1499	1.62
	DEYR 33	1.59
	RIJM	1.50
	LETAN 86	1.40
	ASTRALED 238	1.40
	HANAD 489	1.31
	GULASTRA 521	1.28
	FERSEYN 1381	1.25
	RASEYN 597	1.19
	RUSTEM	1.16
	MIRAGE 790	1.12
	RIFAGE 1286	1.12
	ANTEZ 448	1.06
	RIBAL 397	1.03
	BERK 343	1.00
	ZARIFE 885	1.00
	RAFFLES 952	1.00
	INDRAFF 1575	1.00
	ABU FARWA 1960	1.00
MARES	FERDA 596	1.44
	NARGHILEH	1.19
	RIFALA 815	1.06
	MOLIAH 109	1.03
	RIJMA 346	1.00
	REHASAFA 1269	1.00
	SANKIRAH 149	.97
	FARASIN 615	.94
	JASKOLKA	.94
	MAHROUSSA	.94
	URFAH 40	.91
	GULNARE 278	.91
	ROSE OF SHARON 246	.88
	RISALA	.88
	RIDAA	.81
	RYFKA	.81
	WADDUDA 30	.78
	BENEYEH 614	.75
	INDAIA 813	.75

4. Animals with the highest relationship to the breed during the period 1947-1950 were Skowronek with 2.06 percent, Hamrah with 2.0 percent, Nasik with 1.97 percent, and Mesaoud with 1.72 percent. Females with high relationship to the breed were Ferda with 1.44 percent, Narghileh with 1.19 percent, Rifala with 1.06 percent and Moliah with 1.03 percent.

5. Skowronek, Mirage and their progeny had the highest relationship to show winners. Egyptian female lines produced many of the dams of show winners.

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Gain in Weight of Pregnant Mice in Relation to Litter Size

(*Continued from page* 88)

born by that time the mother was kept under hourly observations and if birth occurred the number of young was recorded. If parturition did not take place by 3 p.m. the mother was killed and the living young were removed from the uterus. Such birth will be referred to as "cesarian." In all such cases the uteri were examined and if in addition to living young partially reabsorbed or dead foetuses were present, the mothers were excluded from the tabulation. In those cases where normal birth occurred it was impossible to know whether reabsorbed foetuses had an influence on the gain in weight. However, in most litter-size groups both normal and "cesarean" delivery were included and a comparison of the two groups showed no significant differences. All single young were born by "cesarian" delivery and the tabulation includes only those mothers in which the uteri contained no dead foetuses. Litters of 8, 9 and 10 young are rare and only a few mothers were available in these groups. The average initial weight of all the animals used was 22.5 grams (variation 18-29 gr.). The average age was 242 days (variation 148-390 days).

In some pregnant females an increase in weight was noted by the fifth day after the plug. All of them showed a gain by the tenth day. However, some increase in weight also occurred in pseudopregnant animals, which mated but did not become pregnant. The weight records of ten such females showed an average gain of about two to three grams between the fifth and eighth day. It is safe to state that if the female's weight-increase continues after the ninth day she is pregnant.

Figure 23 shows the average weight gain of mothers grouped according to the size of their litters. The mother of a litter of five young gains about 2.5 times as much as a mother of one, while a mother of a litter of 10 gains about 3.5 times as much as a mother of one, and about 1.5 times as much as a mother of 5. The total gain in weight is of course greater than the weight of the young produced. It includes also the placenta, amniotic fluid and the weight increase of the mother's own tissues. The dotted lines in Figure 23 show how much gain was contributed by one young in each litter-size group. While a mother of a single young gains 5.5 grams, a single young in a mother gravid with 10 young is responsible for a gain of only 1.9 grams.

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MUTATIONS IN APPLES INDUCED BY X-RADIATION

C. J. BISHOP

Experimental Station, Kentville, Nova Scotia

WHILE the history of X-ray induced mutations dates back to the work of Muller,[9] the application of this artificial type of radiation to the production of new types of horticultural crops has been very limited. When the field of experimentation is narrowed to tree fruits, the amount of experimental work that has been carried out is even smaller, and the only complete reports published to date are those from the Balsgård station in Sweden. The only other center where extensive experimentation has been carried out is at the Canadian Experimental Station at Kentville, Nova Scotia. The work in Sweden was begun in 1944 and that at Kentville in 1948. While in Sweden other mutagenic agents such as neutrons, radioactive phosphorus and sulfur, etc., have been used, the work which has been carried out at Kentville has been entirely with X-rays.

Although the two research centers cited are the only ones that have done extensive work, they are not the only ones that have tried X-ray treatments on fruit trees. Two early unreported experiments by Murneek and Stadler at the University of Missouri and Webster at Macdonald College about 1930-1932 yielded negative results.

About 1939 a number of small apple trees were X-rayed by Nebel[10] using the million volt General Electric X-ray tube in Schenectady, N.Y. The dosages were low, less than 2000*r*, and no report of positive results has been published.

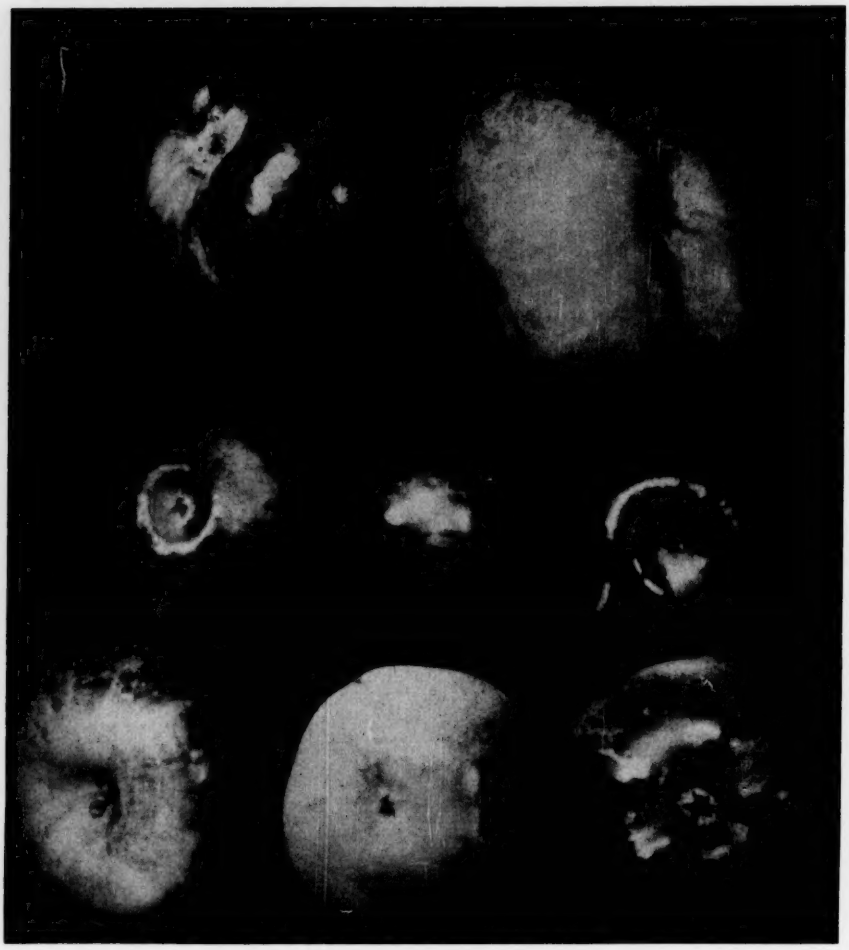
Schmidt[11] noted that a number of varieties of apples were treated with X-rays in Germany in 1938, but were all killed by freezing in the winters of 1939-40 and 1941-42. Additional experiments were started in 1948 but there have been no further reports of this work.

Stadler[13] first pointed out the possible use of X-rays in the development of bud sports in apples, but many others have realized the potential usefulness of this type of radiation in developing new strains of apple varieties.

X-ray work on apples in Sweden was initiated in 1944 by Gustafsson and Nilsson and later was carried on by Granhall[7] at the Balsgård Fruit Breeding Institute. The work was mainly limited to Swedish apple varieties, but also included the varieties Cox Orange, Gravenstein and Ribston which are also grown in Nova Scotia. The progress of this work has been summarized by Granhall,[4, 5, 6] Granhall *et al.*[7] and Ehrenberg and Granhall.[2] X-ray treatments on apples from 1944 to 1951 amounted to 877 scions (Granhall[5]). Recent work in Sweden has been concentrated on other types of radiation and radioisotopes.

From this X-ray work Granhall[6] reported three induced mutations in pears, one a green stripe, one a complete russet, the third a partial chlorophyll deficiency in the leaf, and three color sports in apples of the triploid variety Gravenstein. All three cases showed an increase in the red blush of the fruit color and changes in the prominence of the natural red striping. The fruit were from scions which were treated with 5000*r* of X-rays and grafted in 1948. To establish them as certain mutations they will have to be followed for several seasons.

The dosages used with pears and apples ranged from 500*r* to 25,000*r*, and the 50 percent survival dosage was found to be about 5000*r*. It was also shown[6] that triploid and tetraploid



SIZE AND COLOR CHANGES

Figure 28

Sectorial color chimaeras in Cortland apples from X-rayed scions are shown at the top. Positive change is on the left and negative change on the right. Giant sized, irregularly-shaped apples from X-rayed Cortland scions are shown at the bottom. Normal fruit is seen in the center row.

varieties had greater resistance to irradiation than diploid varieties.

Materials and Methods

Dormant scions of four diploid apple varieties were X-rayed, mostly with a dosage of 3500-4000*r* and the scions grafted by the framework method[8] into grown trees, inserting from 100 to 200 scions per tree.

The X-ray machine was operated at 140 KV and 5MA using a dosage rate of 3-500*r* per minute. Because the irradiation distance was relatively short, there was a variation of about 15 percent above and below the average dosage. Also, as the scions were X-rayed in a layer about 2 cm. thick, there was a differential in dosage from the top to the bottom of the pile. This error was reduced somewhat by overturning the scions and changing their position several times during the X-ray treatment.

The varieties used in the experiment were Yellow Bellflower, Cortland, Sandow and Golden Russet. In none of these are complete natural sports known at the present time, although natural sectorial chimaeras have been found in Cortland and Golden Russet. The parentage of Yellow Bellflower is unknown; Cortland is a cross between McIntosh and Ben Davis, in both of which sports have been found; Sandow is a seedling of Northern Spy, of which several sports are known; and the parentage of Golden Russet is unknown.

The X-ray treatments on the different varieties are summarized in Table I. The number of scions actually grafted is only approximate, as is their survival, although the latter figure is nearly correct. All figures are conservative estimates. In some cases filler scions were grafted into the trees the second year to replace the scions that were killed by the X-ray treatment or died from natural causes.

One advantage of the framework method of grafting is that it forces the scions into early fruiting. It also means that large numbers of scions may be treated, thus greatly increasing the chances of producing a desirable gene mutation. Since this mutation frequency may be in the order of one in several hundred or possibly a thousand buds, the number of scions is important.

Results

Among the morphological effects noted in the early growth from the X-rayed scions were frequent leaf abnormalities and bifurcations of the shoots similar to those reported by Granhall *et al.*[7] Most of the leaf changes were confined to the first few leaves formed, later growth apparently being normal. The shoot bifurcations were usually at a later stage, and in a few cases more than one occurred in the growth from a single bud. The split in the apical growth appeared in some cases in an internodal region, and in other cases was due to the development of a lateral bud. Another fairly frequent occurrence was the death or cessation of terminal growth and a continuation of the growth from the first lateral bud.

The morphological changes were of the type due to chromosome effects from the radiation rather than gene effects, and in general did not persist beyond the first season's growth.

The types of gene mutations which one would expect to detect most readily are color changes in the fruit or possibly chlorophyll deficiencies in the leaves. Economically, the most valuable mutation in apples would probably be color changes in the fruit. The presence of red overcolor has been shown to be due to the action of one or more dominant genes,[14] and so it would be desirable to produce these dominant mutations in varieties lacking in color.

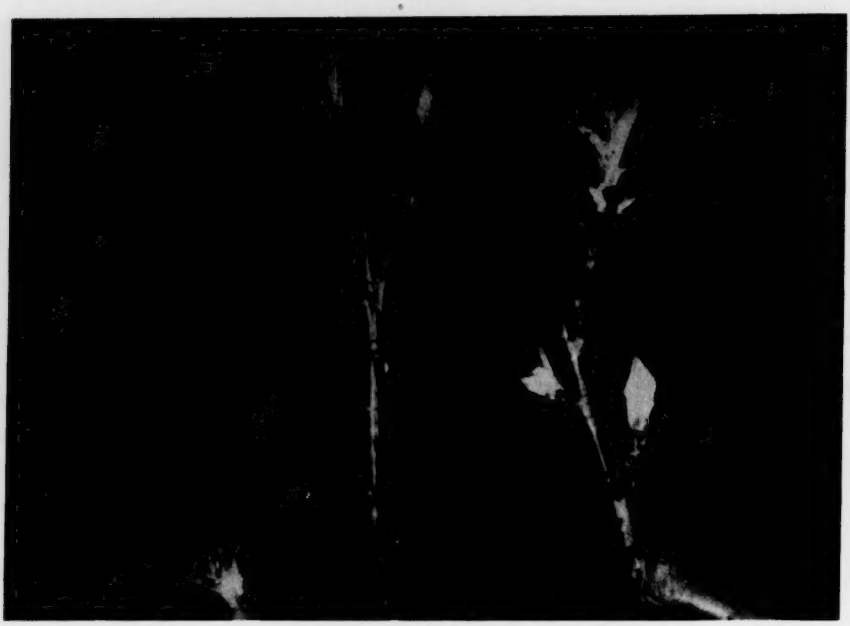
These genes appear to be additive in their effect, so in the varieties which are already partly red, it is still possible to produce darker red sports. In such varieties it is also possible to obtain apples of a lighter color, which is a recessive mutation.

These gene mutations resulting in bud sports probably arise in single meristimatic cells of the shoot meristem and for this reason in most cases are probably confined to a single layer of the plant structure. Einset[3] and Dermen[1] have both shown the nature of development of this structure in the case of chromosomal periclinal chimaeras, and the same must apply to gene changes.

TABLE I. Summary of X-ray experiments with apples, Experimental Station, Kentville, Nova Scotia, 1948-1952

Year	Dosage	Variety	Grafting stock	Scions treated	Number survived
1948	5,000-40,000 *r*	Y. Bellflower	Grown trees	680	Very few†
1949	4,000 *r*	Y. Bellflower	Grown trees	750*	450
1950	3,000 *r*	Cortland	Grown trees	3000	2400
	3,000 *r*	Sandow	Grown trees	500*	350
1951	3,500 *r*	Sandow	Grown trees	500*	350
1952	3,500 *r*	G. Russet	Grown trees	2400	2000
				7830	5550

*Figures include filler scions grafted in the following year to replace dead scions.
†Trees later cut.



SHOOT GROWTH

Figure 29

Normal (left) and abnormal shoot growth in the Cortland apple induced by X-rays.

Thus, to affect the skin of an apple one of the outer layers of the tissue must be involved, possibly the second. A genetic change in one of the internal layers would probably never become visible and its presence would not be detected.

In many cases, we can expect that only a small sector of the layer will show the gene change because of the production of the mutation in an older cell further from the apical primordial region. In such a case, only a portion of the outer layer will be affected, and the result will be a sectorial periclinal chimaera. This will show in the fruit as an apple with a streak of either lighter or darker red color.

The changes so far observed at Kentville have been limited to Cortland, a rather early bearing variety, and the X-ray treatment seemed to induce even earlier blossoming. In this variety a considerable amount of bloom was produced after one season's growth. This was in many cases associated with a short, abnormal, greatly enlarged shoot growth, usually one or two inches in length and about half an inch in diameter (Figure 29). Frequently these abnormal shoots produced flower buds, and the majority of the flowers formed on the Cortland scions in the first year were from such shoots.

The first fruits from any of the X-rayed scions were produced in 1951. They included about a dozen apparently normal apples from the yellow Bellflower scions and somewhat over 200 apples from the Cortland scions. Of the latter, approximately 150 were picked from the trees and carefully examined for color changes.

The most striking change seen in the fruit from the X-rayed scions was that most of them were of giant size and very irregular in shape (Figure 28). A similar effect has been reported in pears from X-rayed scions.[4]

A close examination of the fruit showed a total of nine sectorial color changes (Figure 28) in the 150 apples. Several of these were very distinct and involved as much as one-third of the apple. Changes both in the direction of more and less color were noted in about equal numbers. An examination of 150 non X-rayed Cortlands showed five natural color changes, but in all cases they were minor streaks rather than sectors and their width was very narrow.

In Table II are recorded the number, direction and size of all the sectorial changes discovered, in both the X-rayed and non X-rayed material.

Although more than one sector was

occasionally found on a single apple, in no case were both positive and negative changes found in a single fruit. It is assumed that the several sectors simply represent the variable expression of a chimaeral condition, rather than several mutations, and in Table II the measurements were added together in calculating average widths.

In 1952 a crop of approximately 20 bushels of fruit was produced from the X-rayed Cortland scions, roughly 2500 apples. Of these only two apples were found which were sectorial chimaeras, and these both showed minor changes. All the apples were normal in size. The explanation for the difference between the two year's results seems to be that the first year's production was largely from flower buds induced by the X-ray treatment on abnormal growth. Those producing fruit the second year were largely from unaffected scions, and the spurs producing flower buds the first year became vegetative the second year and outgrew the stunting effect of the first year's abnormal growth. In the case of fruit spurs which produced chimaeras the first year and were marked with tags, no fruits were formed the second year.

The very low frequency of mutations in the lot examined in 1952 would also suggest that the natural mutation rate of four in 150 apples of the previous year may be too high. It is possible that some of the apples of the previous sample were from a branch having a natural sectorial chimaera.

Discussion

The color changes noted in the present work differ from those reported by Granhall[6] in that they are sectorial rather than complete sports. In view of the rather complex apical meristematic growth in the apple branch and the fact that the outer part of the fruit is modified stem tissue, it might be expected that sectorial sports would occur more frequently. This seems to be evident also from a general observation of their natural occurrence. However, the great variety of complete sports[12] would certainly suggest that these also, though rarer, would not be unexpected.

TABLE II. Natural and X-ray induced sectorial color changes in Cortland Apples

Natural			X-rayed		
Apple number	Positive change cm.	Negative change cm.	Apple number	Positive change cm.	Negative change cm.
1	1.0		1	3.0	
	1.0		2	0.6	
2	0.6		3	1.2	
3	0.2		4	4.2	
4	0.8		5	1.6	
5		0.7	6	1.8	
		0.6	7		1.9
					1.6
Average	0.9	1.3			1.3
			8		7.5
			9		1.2
			Average	2.1	4.5

Since sectorial sports occur naturally, some doubt could be raised as to whether those found on the X-rayed scions were actually induced by radiation or were simply naturally occurring ones. In addition to their higher frequency, the best indication that they were induced was their more distinct appearance and greater width. Table II shows that the average width of the chimaeras found on the X-rayed scions was two to three times that of the ones on the non X-rayed trees. None of the natural sectors was found to be over 1.0 cm. in width while the largest on fruit from the X-rayed scions was 7.5 cm. The fact that there were both more and larger sectorial changes in the apples from X-rayed scions would seem to indicate quite conclusively that they were actually induced by radiation.

It is also interesting to note that there were more changes to a darker red color than in the opposite direction. Since it is sometimes considered that mutations induced by X-ray are destructive changes, one would not expect mutations from the recessive to the dominant type, i.e., from green to red. However, these did occur and therefore indicate that either such changes are possible with the use of artificial radiation or that some related mechanism is being affected.

The potential value of these induced sports is naturally the greatest where complete sports are found. However, since a change in a sector of the fruit indicates a corresponding change in a sector of the stem of the branch bearing it, it should be possible, if the bud producing the sport can be propagated, to select or induce buds which show increasing amounts of the colored sector and finally a completely red sport.

On the basis of the results from the two year study it would seem that it might be profitable to attempt to induce more changes by using larger dosages. In the present experiment the scions which were most affected were forced into early production, and it was in the fruit from these scions that the greatest number of changes was found. An increase in dosage would cause a corresponding increase in the death of the scions, the 50 percent survival dosage being about 5,000*r*. However, the increased genetic change might make the technique more worth while.

Summary

Dormant scions of the diploid apple varieties Yellow Bellflower, Cortland, Sandow and Golden Russet were treated with 3500-4000*r* of X-rays and grafted into grown trees by the framework method, inserting 100 to 200 scions per tree.

Typical abnormalities in the leaves and bifurcation of the branches were observed

in the early growth from the scions. The X-rays also caused an abnormal short thick shoot development and induced early formation of the flower buds.

During the second season, grafting these buds produced a total of about 200 apples, many of them of giant size and irregular in shape. Of 150 apples carefully examined when picked, nine were found to be sectorial color chimaeras.

Color changes were observed in the direction of both more and less red color. A comparison of the picked fruit from the X-rayed scions with a sample of 150 apples from non X-rayed trees showed both more and wider sectors in the fruit from the X-rayed scions.

The results indicate that X-rays may be used as an artificial method of inducing color gene mutations in apples.

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◆

FURTHER EVIDENCE CONCERNING CHROMOSOME NUMBERS IN THE PIG

SINCE my manuscript, Chromosome Numbers and Experimental Polyploidy in the Pig, was published in the January-February 1954 issue of the Journal of Heredity I have discovered an article which shows that the chromosome number of $2n = 40$ had been found in the germ line of some varieties of the pig as early as 1917[1]. The later determinations, which have shown that $2n = 40$ is also correct for other varieties of the pig[2, 3], have thus fully confirmed these early counts. It is indeed of interest that the correct chromosome number for the pig of $2n = 40$ had been established by Hance nearly 40 years ago.

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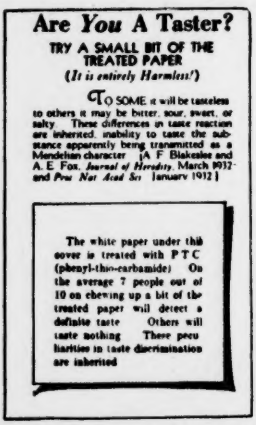
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